SOCIETY FOR NEUROSCIENCE
ABSTRACTS
VOLUME 18, PART 2

22ND ANNUAL MEETING
ANAHEIM, CALIFORNIA
OCTOBER 25–30, 1992
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* 9,379 volunteer abstracts, 18 symposia abstracts, 17 history of neuroscience abstracts and 46 teaching of neuroscience abstracts.
1992 Program Committee

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University of Michigan School of Medicine

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Case Western Reserve University
School of Medicine
# CHRONOLOGICAL LIST OF SESSIONS

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**Decade of the Brain Lecture — 8:00 p.m.**
1. The Human Brain and Behavior: Insights From Modern Imaging Techniques  
   M.E. Raichle ............................................................  No Abstract

## MONDAY, OCTOBER 26

**Symposia — 8:30 a.m.**
2. Emerging Principles of Organization Within the Midbrain Periaqueductal Gray Matter  
   *Chaired by:* M.T. Shipley and R. Bandler ........................ 1
3. The Computational Neuron  
   *Chaired by:* T.J. Sejnowski ............................................. 1

**Special Lecture — 10:00 a.m.**
4. Olfactory Mechanisms in an Insect Model  
   J.G. Hildebrand ............................................................. No Abstract

**Special Lecture — 11:45 a.m.**
5. The Molecular Basis of Electrical Excitability in the Brain:  
   Structure, Gating and Neuromodulation of Sodium Channels  
   W.A. Catterall ............................................................... No Abstract

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   Chaired by: L.D. Fricker ........................................... 381

Special Lecture — 10:00 a.m.
164. Nerve Growth Factor and Nociception
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165. The Biology of Sexual Orientation
   S. LeVay ................................................................. No Abstract

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85. Teaching of neuroscience: elementary and secondary grades

Symposia — 1:00 p.m.

243. New Waves in Cell Calcium
Chaired by: M.R. Hanley

244. The Role of Sensory Information in the Guidance of Voluntary Movement
Chaired by: I. McCloskey and A. Prochazka

Warner-Lambert Lecture — 1:00 p.m.

245. Neuropeptides in Perspective
T. Hokfelt

Special Lecture — 4:15 p.m.

246. Neurotrophic Factors, Their Receptors and the Signalling Pathways They Activate
G.D. Yancopoulos

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**Social Issues Roundtable — 4:30 p.m.**

308. Neural Circuitry and Free Will: Concepts of Responsibility in the Decade of the Brain  
*Sponsored by:* Social Issues Committee of the Society for Neuroscience, H.J. Ralston, Chairperson ........... No Abstract

**The Grass Foundation Lecture — 8:00 p.m.**

309. Molecular Biology of the Glutamate Receptors  
S.F. Heinemann .................................................. No Abstract

**WEDNESDAY, OCTOBER 28**

**Symposia—8:30 a.m.**

*Chairied by:* J.D. Buxbaum and A. Goate ...................... 740

311. Cell Biology of the Growth Cone  
*Chairied by:* D.J. Goldberg ................................. 740
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*Chaired by:* S.A. Lipton

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*Chaired by:* C.A. Barnes
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### THEME E: ENDOCRINE AND AUTONOMIC REGULATION

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**THEME G: MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION**

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**THEME II: NEURAL BASIS OF BEHAVIOR**

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**302. Cognitive and Neurobiological Consequences of Normal Aging:**

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390.3 ADHESION, ANTI-ADHESION AND MIGRATION OF OLFACTORY NEURONS AND NEURAL PRECURSORS ARE REGULATED BY DISTINCT MOLECULAR DOMAINS OF LAMININ. A.J. Calof 1, E.D. Yurchenco 2, J.J. D'Amelio 1, and A.D. Fishman 1 1The Rockefeller University, New York, NY, USA; 2The Johnson Medical Sch., Piscataway, NJ. 2Mass. Inst. of Tech., Cambridge, MA.

Neuronal precursors and immature neurons of the mouse olfactory epithelium (OEC) migrate into vivo, and can be isolated migrated and are monitored in vitro by the ECM protein laminin (LN) and its homologous merosin (LNα3β3γ). LN and LNα3β3γ are also anti-adhesive, i.e., they cause OEC neuronal cells to adhere weakly to substrata that would otherwise be strongly adhesive (Calof and Lander 1991 J. Cell Biol. 115:779). Investigations into the domains of laminin responsible for its anti-adhesive effects on OEC cells have revealed the following: The anti-adhesive activity of LN is highly heat stable and maps to the E1 fragment of the molecule. Although intact uL15 is a cell-surface receptor known to interact with the E3 domain of LN, function-blocking antibodies directed against this integrin do not inhibit anti-adhesion. The migration-promoting activity of LN is distinct from its anti-adhesive activity, and is not the result of adhesion-altering effects of LN. Migration-promoting activity is heat-labile, maps to the E2 fragment of LN, and can be completely blocked by the monoclonal antibody directed against integrin subunit α3. Migration promoted by MN, however, is only partially blocked by this antibody. Surprisingly, although LN is not detectable adhesive for OEC neuronal cells, some of its domains are. E1 is weakly adhesive, and a recombinant (G-domain) (rG) is strongly adhesive. Adhesion to rG was not dependent on α6-containing integrins, nor could it be blocked by a peptide contained in rG that is thought to represent the binding site of integrin α6β1. Adhesion to rG was blocked, however, by low concentrations of heparin (4 μg/ml) and by antibodies directed against LN E3 fragment (which is composed of a G-domain). These data suggest that neuronal adhesion, anti-adhesion and migration can be independently regulated by distinct domains of LN and distinct receptors.

390.5 NEURAL MIGRATION AND LAMININ - STUDIES ON THE ROLE OF LAMININ, THE NEURITE OUTGROWTH DOMAIN OF THE B2-CHAIN, AND CELLULAR MIGRATION AS REVEALED BY IMPARED VIDEO MICROSCOPY. P. Liese 1, E. Trenkner 1, H-U. Dübner 2, W. Siegling 2, Institutes of Biotechnology (Helsinki) and Basic Res. (New York) 1, and Max-Planck-Institut for Psychiatry (Munich) 2.

Low magnification video microscopy on cerebellar neurons growing on a laminin substrate showed that neurons migrated by first sending out a process that contacted the substratum, which was followed by nuclear movement inside a prefixed process. This was also verified on living slices of cerebellum by a novel technique of infrared video microscopy. This technique showed that external granule cells and Purkinje cell bodies attach to the basement membrane and extend out another process towards the presumptive granule cell layer. Thesvesh showed a back- and forth movement that closely resembled that on a laminin substrate. The role of the neurite outgrowth domain of the B2 chain of laminin on granule cell migration was studied in the cable culture system. Neuronal migration in these cultures was totally inhibited by antibodies against this domain laminin. Immunocytochemistry localized this domain in intimate cell-to-cell contacts between the migrating neurons and other neurons and glia. Studies on weaver granule cells on a laminin substrate revealed that these cells were proteolytically more active than their normal counterparts and deposited large amounts of the neurite outgrowth domain generating peptide antigen on their surfaces. These results indicate that neuronal migration on laminin mimulates that visualized in living slices of newborn rodent cerebellum in situ. The B2 chain laminin may be essential for neuronal migration, and as weaver granule cells are proteolytically active, and over express the neurite outgrowth domain of the B2 chain of laminin, the reported neurotoxicity of this domain may provide a mechanism for granule cell death occurring in this mutation.


Neuronal migration in various parts of the central nervous system depends on the motility of a leading process along a radial glial fiber. In order to investigate whether a monoclonal antibody that had previously been shown to inhibit outgrowth of retinal neurites by arresting their growth cones in vivo (Henke-Fahle and Roosheh, Nature 303, 65; 1983) also interfered with neuronal migration, we performed perturbation experiments in living embryos.

Hybridoma producing antibodies against T6 antigen were injected into the menencephalic ventricle of chick embryos at embryonic day 6 to provide a permanent source of antibody. After further incubation (4-9 days) the distribution of antibodies and the thickness of brain sections were examined. The antibodies had penetrated the tectal wall completely, whereas the hybridoma cells remained confined to the ventricular lumen. The observed reduction was discussed in the following way: 1. The neuroepithelial cell layer appeared thicker and consisted of more cells when compared to controls. 2. The Stratum album centrale (SAC) was dislocated towards the pial wall. 3. The Stratum griseum et fibrosum superficiale (SGFS) was drastically reduced in size. These results suggest that young neurons were impaired in their ability to migrate radially towards the future target layers in the presence of T6 antibodies.

Western blot analysis showed two bands of apparent molecular weight 380 and 170 kD. Solubilization properties of the antigen indicated that T6 is not a transmembrane protein.


Cell lineage in the cerebral cortex can be traced using a library of retroviruses that carry distinct DNA inserts as genetic tags. When the tags are analyzed using the polymerase chain reaction (PCR), they mark clones of cells independent of migration patterns of sibling cells. Cortical clones marked in this way often show widespread dispersion, in some cases covering much of the neocortex (Walsh and Cepko, 1990. Science, 245: 431-433). One such clone, that received viral infection at E14-E16 days (E28-21) or 10 days (P3) later can show how this widespread dispersion occurs. Inoculations were made such that 5-50 clones per hemisphere were labeled. The brains were sectioned, reacted for β-galactosidase histochemistry, and labeled cells were plotted using a three-dimensional computerized reconstruction program (CARP). 1990, Development 110: 713). Labeled cells from tissue sections were processed using PCR to analyze the development of the cell types and distribution of neuronal patterns.

Widespread cortical clones were very common after short survival times, at E21, 32% of all cortical clones showed widespread dispersion. At P5, 47% of clones distributed widely. At P30, widespread dispersion in sagittal sections included 22% of all cortical clones. Widespread cortical clones were widely dispersed, accounting for the overwhelming majority of labelled cortical neurons. At E21, there was extensive clonal dispersion in the neo-caudal or antero-lateral direction, and clones included cells in the cortex and the proliferative zones. This suggests movement of proliferating cells essentially parallel to the ventricle. At P3, dispersion in the medial-lateral plane was greater than at embryonic stages, which may reflect dispersion of pontine neurons into the lateral ventricle (Reillo, L., 1991, Soc. Neurosci. Abst. 17:533). Thus, widespread clonal dispersion reflects several phenomena occurring in sequence. Supported by the Howard Hughes Medical Institute and the NIMH.
390.9 LATERAL DISPERSION OF PREMIGRATORY, NEURAL PROGENITORS WITHIN THE VENTRICULAR ZONE OF CEREBRAL CORTEX: O. Finnila, C.A. Mason and M.E. Hatten. Dept. of Pathology in the Center for Neurobiology, College of Physicians and Surgeons, Columbia University, New York, N.Y., 10032.

In cortical regions of developing brain, neural precursors are generated in compact ventricular zones along the inner surface of the neural tube, after which postmitotic progeny migrate along the glial fiber system to establish the cortical laminae. Although recent evidence suggests significant dispersion of clonally related neurons during development, the relative contribution of movements within the germinal zone and tangential movement across the radial glial fiber system have not been determined. To visualize the dispersion of neural progenitor cells within the ventricular zone of the mouse cerebral cortex, the lateral wall of the telencephalic vesicle was removed on embryonic day 15, and a random population of cells on the ventricular surface was labeled with a dilute solution of the lipophilic dye Dil. Immunostaining with the neural antigen nestin and with BrdU demonstrated that labeled cells were within the VZ. By correlated video-enhanced, fluorescence and phase-contrast microscopy short bursts of motility at distances between 10-100μm were rapidly dispersed labeled cells across the ventricular zone. Labeled, dividing cells gave rise to daughter cells which separated and moved independently across wide areas of the cortical VZ. To determine whether cell dispersion was confined to the cortical VZ, we examined the behavior of labeled cells approaching the boundary with the lateral ganglionic eminence. Labeled cells did not cross from one proliferative zone to the other. Instead they contacted the boundary with filopodial extensions and moved retro-caudally along the interzone. These boundaries separate and maintain a regional pattern of neurogenesis in developing brain. Within the germinal zone of the cerebral cortex, neural progenitors disperse widely prior to radial migration along the glial fiber system.

390.10 RESCUE OF WEaver GRANULE NEURON DIFFERENTIATION BY TRANSLATION OF THEIR PROGENITORS INTO WILD-TYPE DEVELOPING CEREBELLAR CORTEX. W.-Q. Gao* and M.E. Hatten. Dept. of Pathology, Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

The migration of postmitotic neurons away from compact, germinal zone in developing brain is a critical step in CNS neuronal differentiation. To examine the autonomy of expression of molecular signals for cerebellar granule cell migration in vivo, neuronal progenitors purified from the neurological mutant mouse weaver, an animal with phenotypic defects in migration, were transplanted into the external germinal layer (EGL) of wild-type developing cerebellar cortex. In wild-type cerebellar cortex, dye-labeled, weaver EGL progenitors progressed through all of the classical stages of granule neuron differentiation, including the extension of parallel fibers, migration through the molecular and Purkinje cell layers, final positioning in the inner granule cell layer, and extension of dendrites. These observations provide evidence that weaver gene acts non-autonomously in vivo, and suggest that local interactions in the cerebellar EGL induces initial steps in neuronal differentiation required for granule cell migration.


In order to compare the mechanism of neuronal migration with axon extension in vitro, granule neurons were purified from early postnatal cerebellar cortex (P4-P6) and cultured with the fluorescent lipophilic dye, Fki-26. Labeled neurons were co-cultured with unlabeled astroglial cells and neuronal migration was examined by a combination of time-lapse video and fluorescence microscopy using a light-automated CCD camera. Examination of labeled neurons revealed frequent extension and retraction of the leading process during migration, as well as lamellopodial and filopodial extension along the entire length of the leading process. The dynamics of leading process motility differed from that of the growing granule neuron axon, where motility was confined primarily to the growth cone, which moved forward steadily without retraction. These observations suggest that neuronal migration in vivo occurs along the lamellipodia and filopodia extension along the entire length of the leading process.

New PET studies have demonstrated the sensitivity of [11C]-methylpyropropioniofuran (a high affinity D2 dopamine ligand) binding to pharmacologic alterations in endogenous dopamine (Dewey et al., 1990, 1991, Logan et al., 1991). The present study was undertaken to further examine drug-induced changes in dopamine using [11C]-raspipride, a lower affinity D2 ligand, and two drugs which increase dopamine by different mechanisms. PET studies were conducted in anesthetized, adult female baboons (Papio anubis) using the CT1931 tomograph. Two [11C]-raspipride scans were performed, two hours apart, prior to and following drug intervention with either d-amphetamine, which releases cytosolic dopamine (1.0 mg/kg, IV, 5 minutes pre-injection) or CH-22909, the selective dopamine reuptake inhibitor (1.5 or 3.0 mg/kg, IV, 20 minutes pre-injection). The data were analyzed with the distribution volume method (Logan et al., 1990). After both interventions, [11C]-raspipride binding was decreased, bilaterally, in the striatum (specific binding), but not in the cerebellum (non-specific binding). These decreases exceed the test-retest variability in the same animals. The rate of metabolism of the radiotracer was unaltered. Therefore, [11C]-raspipride binding is sensitive to alterations in endogenous dopamine. This finding provides further support for the utility of PET in assessing the functional state of the dopamine system, in vivo. Supported by DOE/OHER, NIH: NS15638, NS15380.


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391.2 SIMILARITIES AND DIFFERENCES IN DOPAMINE RECEPTOR SUBTYPE REGULATION: MOLECULAR MECHANISMS. Ian Greenspan and S.-X. Xu, Center for Molecular & Behavioral Neuroscience, Rutgers-Newark, NJ 07102.

Steady state levels of cell surface receptors are determined by many distinct processes, each of which may be subject to different regulatory mechanisms. Both striatal D1 and D2 receptors show a similar developmental pattern where receptor number increases by 6-14 fold over the first 30 days postnatal. However, mRNA levels for both receptors increase by only 2 fold over this period. Whereas denervation, by a 6-OHDN lesion of the nigro-striatal pathway, increased D2A receptor mRNA levels by 53% and receptor level by 38%, D1A receptors and their mRNA were unchanged. In contrast, a 3 week administration of the D2 receptor antagonist SCH23390 (0.5mg/kg/day, s.c.) increased both D1A receptor density and mRNA levels. However, treatment with the D2 receptor antagonist haloperidol (0.5 mg/kg/day, s.c.) had no effect on striatal D2A mRNA levels, in spite of significant increases in receptor Bmax. This was not the result of the intermittent receptor blockade following drug injection, as chronic infusion of haloperidol via osmotic mini-pump also did not increase D2A mRNA, in spite of a 59% increase in receptor Bmax. Interestingly, chronic dopamine depletion with reserpine did not affect mRNA levels for D2A or D2A receptors, with only D2A receptors being significantly upregulated. These findings indicate that the various molecular mechanisms involved in receptor regulation (gene transcription, receptor degradation etc.) are differentially involved in the regulation of each dopamine receptor subtype. Supported by grants from the Stanley Foundation and NIMH.
**391.3** INCREASED S-HT2 AND NK-1 RECEPTOR BINDING ASSOCIATED WITH SEROTONIN/STEROID SUBSTANCE P HYPERSEROTONINERGIC IN RAT INFERIOR OLIVE. M. Pardé, I. Descaurier* and R. Quinton. Centre de recherche en sciences cognitives (Département de psychiatrie), Université de Montréal, and Douglas Hospital Research Center and Department of Psychiatry, McGill University, Montréal and Verdun, Quebec, CANADA. Serotoninergic/S-HT2 and substance P/NK-1 receptors were measured by ligand binding autoradiography with [3H]ketanserin and [125]I-SH-Sp, in the inferior olive of adult rats previously submitted to cerebroventricular or administration of 5,6-dihydroxytryptamine. This experimental treatment was previously shown to be followed by marked S-HT hyperactivity in most subdivisions of IO (e.g. threc the normal number of S-HT axons variates in the lateral dorsal accessory olive, lat DAO). It is also known to induce a parallel augmentation of the density of SP immunoreactive fibers in several parts of IO, which suggests a co-localization of both transmitters in the same hyperinnervating terminals. In normal IO, the density of S-HT2 sites was relatively low and rather homogeneous. NK-1 binding appeared denser and more heterogeneously distributed. After S-HT1/SP hyperinnervation, considerable increases in the density of both classes of binding sites were observed. Specific [3H]ketanserin binding was now strongest in IO subnuclei, including some in which it had not been detected in the normal. [125]I-SH-Sp binding showed even greater elevations or became detectable in a few subnuclei, remaining unchanged in others, and was slightly decreased in the lat DAO. The normal and altered distributions of both ligands did not match the respective patterns of S-HT and SP innervation and hyperinnervation. In view of the current information on the cellular localization of S-HT2 and NK-1 receptors in IO, it seems likely that both compounds, in the hyperinnervated state, were largely the result of an up-regulation, and not the mere reflection of an augmented number of autoreceptors on 5-HT and/or SP terminals. (Supported by MRC grants MT-3544 and MA-8580)

**391.5** AGE-DEPENDENT REGULATION OF CORTICAL AMINO ACID RECEPTORS. B.A. Lanus* and C. Shaw, Dept. of Neuroscience, Ophthalmology and Physiology, University of British Columbia. We have used a rat neocortical slice preparation to examine the regulation of GABA(A) (PH2-SR 95631), AMPA (PH2-CNQX), NMDA (PH2-2CS9 39653) and kainate (PH2-kainate) receptor populations during postnatal development. Regulation of these receptor populations was achieved using either stimulation with the appropriate agonist or using veratridine and glutamate (V/H+). In adult cortex, V/H+ resulted in an up-regulation of GABA(A) receptors in contrast to a down-regulation of AMPA, NMDA, and kainate receptors. During postnatal development, V/H+ showed age-dependent effects; in animals younger than 25d, GABA(A) receptor number was decreased whereas AMPA receptor number was increased; NMDA and kainate receptors decreased after 400 V/H+ treatment led to an increase in GABA(A) receptor number; at ages greater than 60 d AMPA receptors decreased. NMDA and kainate receptor showed decreases to V/H+ treatment led to down-regulation of all four receptor populations. Quisqualate stimulation of AMPA receptors increased down-regulation with increasing postnatal age, an opposite effect to that observed for GABA(A) and kainate receptors. The differing effects with age on receptor regulation for either changes in cell electrical activity or agonist stimulation may suggest a role for such age-dependent receptor regulation in the events leading to neocortical critical period plasticity.


Based on the nucleotide sequence of rat NMDAR1 (Montal and et al., Nature 354, 870-875, 1992) and the corresponding (S) oligodeoxyribonucleotides (D oligos) in an attempt to suppress synthesis of the NMDAR1 receptor protein. Herein described data were obtained with antiseroltemocholesterol and matching S 18-mer D oligos. These D oligos were added under serum-free conditions to primary cultures of rat cortical neurons which were studied with respect to: (1) H-MK-601 binding; (2) basal and NMDA-induced dehydrogenase (LDH) release; and (3) basal and NMDA-induced Ca**2+** influx. A reduction of %MK-601 sites by 40-50% (vs. S or D oligo) could be achieved by 3-5 days incubation of the neurons with 1 μM of the AS D oligo; this down-regulation was less than that with an antisense S D oligo of the NPY Y1-receptor (Yee et al., this meeting). The AS D oligo induced a concentration dependent (0.1-10 μM) reduction of spontaneous LDH release (measured 1-3 days after removal of serum) and evoked release (5 min exposure of 9-day old cultures to 100 μM NMDA). Finally, we found that spontaneous, but not NMDA-induced, Ca**2+** influx (measured over 2 min) was elevated, up to 2-fold, in neurons exposed to AS D oligos, possibly also reflecting increased degrees of neuronal survival in these cultures. However, there may exist a "window" of optimum AS D oligo concentration, since the S D oligo, at 10 μM but not at 1 μM, showed minor effects per se. In conclusion, the present study indicates the usefulness of the antisense approach for down-regulation of (receptor) protein, such as the NMDAR1. I also illustrate that excitation through activation of the NMDA-receptor is associated with neuronal cell death.

**391.8** ANXIOLYTIC AND ANTICONVULSANT DOSES OF ABBEARNIL FAIL TO INDUCE TOLERANCE AND DEPENDENCE IN MICE AND CATS. M. Serra, G. A. Ghiass, A. C. Padda, C. F. Moir and G. Biggio, Department of Experimental Biology, Chair of Pharmacology, University of Catania, Italy. In mice, [S]TPBS binding and exploratory motility were dramatically reduced by the acute administration of abbearnil (AB) (0.2 - 1 mg/kg i.p.). Correspondingly, the administration of this drug (1 mg/kg 3 times a day) induced in two weeks a significant increase in [S]TPBS binding in the mice cerebral cortex with no change in motor behaviour. This biochemical effect was paralleled by the failure of a challenge dose of AB (1 mg/kg) to modify both [S]TPBS binding and motor activity. On the contrary, in mice chronically treated with a lower but pharmacological effective dose (0.2 mg/kg) the exploratory motility was reduced (95%) by a challenge dose of AB (0.2 mg/kg). This evidence may suggest that tolerance develops only with very high doses of AB. This conclusion is consistent with the finding that in brain of this chronic treated mice the binding of the AB homologous (AB) was unchanged or even increased. Moreover, while in mice treated with the high dose of AB (1 mg/kg) the binding of [S]TPBS was altered after drug discontinuation (increased by 26% at 48 h and decreased by 15% at 96 h), we found no change in mice chronically treated with 0.2 mg/kg at the same times. Our data indicate that chronic administration of AB is associated with the development of tolerance and with discontinuation syndrome only at very high doses while it is devoid of these effects at lower, but pharmacologically effective, doses. This conclusion is also consistent with the finding that in cats chronically treated with AB (7 mg/kg 3 times for 15 days) the interperso nal association of 3-methyl (20 mg/kg) to precipitate an abstinence syndrome.

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391.0 EFFECTS OF LESIONS OF THE DORSAL RAPHE NUCLEUS ON SEROTONIN TRANSPORTER, 5-HT4, AND 5-HT3 BINDING SITES IN RAT.

Decreases in the serotonin (5-HT) transporter and increases in 5-HT1A binding are found in restricted cortical areas of suicide victims. We sought to determine whether 5-HT binding follows lesions of the dorsal raphe nucleus (DRN) in intact rats. 5-HT binding changes reported in man and to examine the extent of any subcortical changes. Tissue samples (corpus striatum, hippocampus, DRN) were counted in 25 regions. 5-HT binding was measured by in vivo autoradiography using [3H]ketanserin, [3H]iminobenzamide, and [3H]ritanserin. DRN lesions were made by stereotaxic injection of ibotenic acid into the nucleus (n=8). Controls (n=6) received 1 mg of 1% ABD. DRN lesions reduced 5-HT binding in all regions examined from 90% (medial prefrontal cortex) to 29% (cingulate/parietal cortex). 5-HT binding was greatest in prefrontal cortex (101±14%) and least in the caudate nucleus (14±3%). DLN lesions reduced 5-HT binding to 5-HT1A binding with increases in frontal cortex (19% of control, p=0.06), intermediate corpus (175%, p=0.09), and hippocampus (113%, p=0.001). 5-HT ketanserin binding ratio from 314±11 binding (control cortex) to 251±15 binding (corpus callosum) and was unaffected by DRN lesions (p=0.05) in any brain region. We conclude that DRN lesions produce: 1) preferential effects of 5-HT projections to the forebrain and brainstem; 2) reversed changes in [3H]DPAT binding and 3) no effect on [3H]ketanserin binding. These findings parallel those in suicides in several respects: 1) 5-HT transporter binding is reduced; 2) 5-HT1A binding is increased; and 3) 5-HT-ketanserin binding is unchanged. Notable differences include: 1) widespread vs. localized reductions in transporter sites and 2) marked reduction vs no change in 5-HT1A and 5-HT1A. The cortical 5-HT receptor changes in suicide are consistent with a partial lesion of the DRN. Supported by NARSAD award to MDU and MH64745.


Considerable evidence has accumulated for neurotransmission dysfunction in the pathophysiology of major depression. One such line of evidence is the reduction in the density of binding sites for [3H]anipramine or [3H]paroxetine, markers of the 5-HT1A post-synaptic receptor, in select areas of patients with major depression. In an attempt to determine whether changes in platelet [3H]paroxetine binding represents changes in CNS 5-HT function, we investigated whether depletion of 5-HT and 5-HT image analysis. 5-HT 5-HT-5HIAA content in frontal lobe were determined by HPLC. In controls, specific 5-HT transporter binding (measured by competition with [3H]DRN [49:16], free binding, lox, of the corpus callosum (831±1) and not differ from sham-operated controls (p=0.05). DRN lesions reduced 5-HT and 5-HIAA by 78% and 92%, respectively, and reduced (p=0.05) transporter binding in all regions examined from 90% (peripheral cortex) to 29% (cerebrospinal fluid). 5-HT binding was greatest in prefrontal cortex (101±14%) and least in the caudate nucleus (14±3%). DLN lesions reduced 5-HT binding to 5-HT1A binding with increases in frontal cortex (19% of control, p=0.06), intermediate corpus (175%, p=0.09), and hippocampus (113%, p=0.001). 5-HT ketanserin binding ratio from 314±11 binding (control cortex) to 251±15 binding (corpus callosum) and was unaffected by DRN lesions (p=0.05) in any brain region. We conclude that DRN lesions produce: 1) preferential effects of 5-HT projections to the forebrain and brainstem; 2) reversed changes in [3H]DPAT binding and 3) no effect on [3H]ketanserin binding. These findings parallel those in suicides in several respects: 1) 5-HT transporter binding is reduced; 2) 5-HT1A binding is increased; and 3) 5-HT-ketanserin binding is unchanged. Notable differences include: 1) widespread vs. localized reductions in transporter sites and 2) marked reduction vs no change in 5-HT1A and 5-HT1A. The cortical 5-HT receptor changes in suicide are consistent with a partial lesion of the DRN. Supported by NARSAD award to MDU and MH64745.

392.1 AMPULPATEAT PECATION OF GROWTH HORMONE (GH) IN CHRONIC RENAL FAILURE. J.D. Veldhuis, M. Willkowitz, A. Iwama.
W.K. Bolotin, Dept. Internal Medicine, NSF Science Center in Biological Timing, Univ. Washington, Seattle, WA 98195, and Salem Veterans Affairs Hospital, Salem, VA 24153.

Uremia evokes alterations in multiple neuroendocrine axes, including the gonadotropin, corticotropin, and somatotropin (GH) axes. We have studied the neuroendocrine control of episodic GH secretion and simultaneously estimated GH half-life in 7 middle-aged men (mean ages 39 ± 5 years) with endstage renal failure as compared to a group of 7 age-matched controls (mean ages 42 ± 4 years). Multiparameter deconvolution analysis (ENAS 84:7953-90, 1987) of 24-hour serum immunoreactive GH concentrations revealed a calculated half-life of endogenous GH of 21 ± 18 min in the men with ESRF compared to 17 ± 20 min in controls. Uremia was accompanied by an increased frequency of pulsatile GH release namely 16 ± 1.0 secretory bursts/24-hr in uremic patients vs 36 ± 11 in the men with ESRF. The mass of GH secreted per burst was increased in uremia (45±1.2 ng/mL versus 30±1.1). Consequently, the 24 hour endogenous GH production rate was approximately 2-fold higher (73±1.8 ng/kg/24-hr) in uremic patients vs 36±11 ng/kg/24-hr in age-matched controls. In contrast, the duration of GH secretory bursts was not altered in uremia. The mean 24-hr serum GH concentration was 1.5±0.38 (uremia) versus 0.62±0.28 ng/mL (control). In summary, uremia is accompanied by a small increase in the half-life of putatively intact immunoreactive GH as well as an increased frequency and mass of pulsatile GH secretion. We therefore hypothesize that somaostatin withdrawal, and to a lesser extent decreased GH clearance, contribute to elevated plasma GH concentrations observed in uremia in humans.

392.2 PATCH CLAMP ANALYSIS OF GROWTH HORMONE-RELEASING FACTOR (1-44) EFFECTS IN HUMAN GROWTH HORMONE SECRETING CELLS IN VITRO. M. Chen, L. Haynes, L. McConnell, J. Cummings and J. C. Clarke. Prince Henry's Institute of Medical Research, P.O. Box 152, Clayton (Melbourne), Victoria 3168, Australia.

We have previously reported that local application of human growth hormone-releasing factor 1-44 (hGRF) in rat pituitary somatotropes causes a depolarization of cell membrane and growth hormone (GH) secretion that depends upon Ca2+ (Chen et al., Neuroendocrinology 50:679, 1990). This effect can be obtained by conventional micropipette intracellular recording but not by standard whole-cell patch-clamp recording. Alternatively in the present experiment, we used the indirect patch-clamp depolarization technique in the whole-cell configuration, this effect has been observed in identified human growth hormone (GH) secreting tumour cells from acromegalic patients. With local application of hGRF (100 nM), 30 sec, we observed depolarization occurs with the generation of multiple action potentials. Current-induced depolarization in the absence of hGRF causes a single action potential but in the presence of hGRF, multiple action potentials are seen. The characteristics of the action potentials, in terms of Ca and Na flux, are similar to previous seen in rat somatotropes (Chen et al., Life Sci. 48:593, 1990). In particular, hGRF increased the action potential due to Ca influx, the Ca current was enhanced by hGRF in cells treated with TTX (20M) and TTX (1uM) in the bath solution and Ca in the electrode solution to eliminate K and Na currents. These Ca currents were also modified by hGRF application. These results show that hGRF can increase influx of Ca in human tumour derived somatotropes via its effects on Ca channels in a manner similar rat somatotropes. Supported by H & MRC and El Lilly Growth Grant.

392.3 INHIBITORY REGULATION OF PULSATILE GHRH RELEASE FROM GT1-1 GHRH CELL LINES BY VASOPRESSIN AND GABA. B.J. Weiger and O. Martinez de la Escalera. Reproductive Endocrinology Center, University of California San Francisco, CA 94143, and Instituto de Investigaciones Biomédicas, National University of México, México City 04510, México.

Arginine vasopressin (AVP) containing nerve terminals synaptic with GHRH neurons and may play a role in stress-induced inhibition of LH secretion. y-aminobutyric acid (GABA) neurons also synapse with GHRH neurons and substantial evidence support their role on GHRH release. GHRH cell lines (GT1-1) release GHRH with a pulse frequency similar to that observed in castrated rats. Supersufusion of GT1-1 cells with AVP (100 mM) inhibited pulsatile release of GHRH. The mechanism by which AVP inhibits GHRH release is not clear since 10-100 nM AVP (110) did not inhibit tyrosine phosphorylation (assayed by phosphorysene Western blots) nor adenylate cyclase activity (assayed by RIA of intracellular cyclic AMP) in GT1-1 cells. GABA (10 mM) did not show a biphasic effect on GHRH release, with a brief and rapid stimulation followed by a long and sustained inhibition. The effect of GABA is correlated with a bicuculline- and saclofen-sensitive inhibition of the basal and stimulated intracellular levels of cAMP. These findings demonstrate that two putative inhibitory neurotransmitter in the regulation of GHRH release are capable of exerting their actions directly on GHRH neurons. (Work supported by NIH Grant HD 08924 and The Rockefeller Foundation).

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Reports of increased immunoreactivity (IR) for enkephalin (ENK) (Soc. Neurosci. Abs. 15:702.1989) and neuropeptide Y (NPY) (Endo.128:823, 1991) in TIDA neurons of lactating rats with an increase in prolactin (PRL) levels in the milk of the same animals (Podor et al. 1992) led us to investigate whether the expression of peptides in the median eminence (ME) is altered during lactation in rats and mice, and whether PRL receptors (PRLR) might be involved in these changes. IRs for NPY, ENK and neuropeptide (NPY) (1991) in TIDA neurons of lactating rats were increased and the NPYergic fibers are unaligned, suggesting that the PRLR might be involved in these changes. IRs for NPY, ENK and neuropeptide (NPY) levels in the ME were dramatically increased in the ME of post-pubertal (PP) day 8 lactating mice that were pup-deprived for two days, compared to continuously nursing (PNP) mice. Fluorescent double-staining showed colocalization of these IRs in all tyrosine-hydroxylase (TH)-IR endings. Preliminary ultrarstructural studies showed that the IRs are a part of the same cell, suggesting a specific function of these neuropeptides among neurosecretory vesicles in the same nerve terminals. On PNP, NPY and ENK IRs were visible in putative ME-TIDA endings of both intact and PPN-injected lactating rats. Changes in NPY-IR could not be detected. Considerable interlaminar variation in NPY-IR suggested the influence of the underlying paracrine phenotype. When intact lactating rats were separated from their litters for four hours and then reunited, we detected a trend for increased NPY-IR, but not for ENK or NPY-IR, in the ME during the 50-60 min following the onset of suckling, associated with elevated circulating PRL levels. NPY-IR, but not ENK- or NPY-IR, was also expressed in putative TIDA endings in orchidectomized rats maintained on estrus. Changes in PNP-IR contain insulin-like growth factor (IGF) in hypothalamic cells of the same animals. These results suggest that 1) an ovarian influence may not be required for the expression of NPY- and ENK-IRs in the TIDA neurons during lactation, 2) PNP may be responsible for inducing NPY expression in these cells, and 3) additional factors may regulate expression of ENK- and NPY-IRs in TIDA neurons with both sex and species differences in their actions.

392.9 SUPPRESSED TYROSINE HYDROXYLASE (TH) AND INCREASED NPY GENE EXPRESSION IN THE ARCULATE NUCLEUS OF LACTATING RATS. H. Li, Wang and M.S. Smith. Department of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

The suckling stimulus alters hypothalamic function, resulting in suppressed GnRH and increased prolactin secretion. To understand mechanisms responsible for these effects of suckling, we assessed whether lactation was associated with changes in gene expression in the arcuate nucleus (AN). Animals were studied during estrus-1, and on day 10 postpartum with 8 pups suckling for 24 h after pup removal. In situ hybridization of AN was performed to assess mRNA levels for TH and NPY. For single label ISH, TH and NPY riboprobes were labeled with 35S. For double label ISH, TH riboprobe was labeled with 35S and NPY riboprobe with digoxigenin. TH mRNA (graft area/AN area) levels in lactating rats were suppressed to 60% in the rostral AN and were undetectable in the remainder of AN, compared to control lactating controls. In contrast, TH mRNA in the zona incerta did not differ between the two groups of animals. At 24 h, TH mRNA levels had increased to nearly 200% of controls. NPY mRNA levels were similar in diestrous and lactating animals except in the caudal area of the AN (at the level of the dorsomedial hypothalamic nucleus) where mRNA levels increased nearly two-fold in lactating rats. The change in NPY gene expression was reversed 24 h after pup removal. Using double label ISH, we found TH and NPY expressing neurons in close proximity in addition to TH positive, but NPY negative neurons for expression of TH and NPY in the same neurons. In summary, the suckling stimulus changes gene expression in specific subpopulations of dopamine and NPY neurons in the AN. These changes appear to be mediated by a change in the dynamics of the transmitter released. Supported by Grant HD14643.

392.10 FOS-RELATED ANGIOTENSINS AS MARKERS OF BASALINE NEURONAL ACTIVITY: EFFECTS OF LACTATION ON ARCULATE NUCLEUS DOPAMINE NEURONS. G.E. Hoffman*, W.J. Lee, A. Abd and M. S. Smith. Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

In many areas of the brain, neurons express Fos-related antigens (FRAs), but not cFos, under baseline conditions. These studies assessed whether FRA expression can serve as a marker for both decreased as well as increased basaline neuronal activity. To test this hypothesis, we examined FRA expression in tuberoneuroblastoplastic (TH) dopaminergic [DA neurons of the arcuate nucleus (ISH)] in cycling female rats and in pregnant and lactating animals. The TIDA neurons comprise the principal prolactin releasing system. TH neuron activity is known to increase in lactating rats, whereas DA neuron activity is decreased. Therefore, the suckling stimulus is a likely mediator of decreased TH neuron activity. This hypothesis was tested in the lactating rat, TIDA neuron activity is presumably inhibited, thereby facilitating prolactin release. Double label immunocytochemistry localized tyrosine hydroxylase (TH) and either FRAs or c-Fos. FRAs and c-Fos were distinguished using double antibodies generated against the common epitope region of FRAs (capable of recognizing most FRAs except c-Fos) and the c-terminal of c-Fos (selective for c-Fos). Under these conditions, c-Fos was expressed in the TIDA neurons. Animals were studied at 0-4 h after pup removal and at 24 h after pup removal and were restored to full expression by 24 hours. The changes in FRAs expression in the presence of the suckling stimulus and upon pup removal parallel changes we observed in TH gene expression in TIDA neurons of lactating animals. These data suggest that FRA expression can effectively serve as a marker of baseline activity in tonic neurons of the brain.
392.11
Rapid changes in oxytocin and vasopressin mRNA concentrations in the early postpartum period: evidence for a role of factors other than suckling. R.S. Crowley and J.A. Amico. University of Pittsburgh School of Medicine and VA Medical Center, Pittsburgh, PA 15261.

Oxytocin (OT) and vasopressin (AVP) gene expression are enhanced in the rat hypothalamus during gestation and during the second and third weeks of lactation. Increased OT gene expression during lactation is believed to be due to suckling, which is known to activate OT neurons resulting in coordinated stimulation of pituitary activity of the OT and AVP neurons and release of OT and AVP from the posterior pituitary. We hypothesized that other factors such as ovarian steroids might modulate responses to stimulation of these neurons. We therefore performed Northern analysis and in situ hybridization during the first ten days of lactation when rats are continuously suckling but ovarian steroid concentrations are known to change markedly. We report that during the first three postpartum days, OT and AVP mRNAs decreased dramatically reaching less than one fifth of peak gestation levels by day 2 postpartum in both suprachiasmatic (SCN) and paraventricular (PVN) nuclei. OT and AVP mRNA returned to levels comparable to late gestation on or about day 10 lactation, and remained elevated after 15 and 23 days of lactation. We have also compared OT mRNA isolated from lactating day 3 rats to cohorts which did not litter at the time of parturition. Lactating rats had significantly lower OT mRNA levels than their non-lactating cohorts. These data refute the hypothesis that lactation itself is not a factor in the increases in OT and AVP mRNAs produced as a result of continuous stimulation by suckling.

392.12
Oxytocin (OT) and vasopressin (AVP) gene expression in the osmotically-stimulated female rat. J.A. Amico, R.S. Crowley, S.M. Chalmers. Univ. of Pittsburgh Sch. of Med. and VA Medical Center, Pittsburgh, PA 15261.

Our finding that ovarian steroids, estradiol (E2) and progesterone (P), modulate hypothalamic OT and AVP gene expression in the lactating rat, led us to question whether similar effects occur in the osmotically-stimulated female rat. Sustained hyperosmolality (4-14 days of oral 2% NaCl) is reported to enhance OT and AVP mRNA concentrations in intact male rats. Similar studies have not been done in female rats. If E2 and P do influence OT and AVP mRNA expression in the osmotically-stimulated female rat, does hyperosmolality enhance AVP gene expression, as observed in the male, or does it decrease it? We have begun studies to address these questions. The responses of these genes to sustained hypernatremia in the female rat would likely be heterogeneous because of the differences between E2 and P in their actions as well as in their levels during the estrous cycle. More upregulation of OT and AVP gene expression should not occur in salt-loaded ovariectomized (OVX) rats. Adult female cycling Sprague-Dawley rats matched for age and weight were sham OVX (intact) or OVX (3 wks prior to study), administered oral 2% NaCl or tap H2O, and sacrificed on the 5th day of study. Salt-loaded OVX and salt-loaded intact rats developed comparable degrees of hypernatremia, depletion of posterior pituitary stores of OT and AVP, and weight loss (no significant differences, ANOVA). At sacrifice, E2 and P concentrations were non-detectable in OVX rats and varied among intact rats, depending upon the stage of the estrous cycle. Salt-loaded OVX rats (n=8) showed no upregulation of OT or AVP mRNAs, whereas salt-loaded intact, cycling rats (n=12), showed both up-regulated OT mRNA as well as decreased AVP mRNA expression. These data and others suggest that ovarian steroid-mediated mechanisms for up-regulation of OT and AVP gene expression are present during salt-loading.

TRANSPLANTATION I

393.1

Implanted chroaffin tissue alleviates some parkinsonism symptoms in humans, but not in experimental animals. Functional recovery could be due to the release of large amounts of both NE and DA from this tissue. Implantable biodegradable controlled-release microphreses containing NE or DA provide a novel means for the sustained restitution of subnormaal DA function. Rats were unilaterally 6-OH-DA lesioned in the MFB. Six to eight weeks later, a suspension of 3ml of DA- or NE-containing microphreses was implanted in 2 rats in each of 2 DA denervated striata. Contralateral rotational behavior induced by apomorphine was used as an index of lesion success and, following implantation of the microphreses, also as an index of functional recovery. Intact rats and NE microphre implanted rats displayed a 30-50% reduction in the number of apomorphine-induced rotations up to 6 weeks postimplantation. Following conclusion of the studies, immunochemical examination revealed growth of DA and tyrosine hydroxylase IR fibers in the striatum of DA and NE microphre implanted rats. Preliminary EM studies showed signs of axonal growth cones and of axonal sprouting in the vicinity of the injected microphreses. Thus, both microcapsulated NE and DA have the capacity to assure functional recovery and to promote DA fiber (re)growth in parkinsonism. This novel means to deliver these substances to the CNS could be of therapeutic usefulness in Parkinson’s disease.

393.2

Polymeric cell encapsulation may be a useful technique for investigating the mechanisms by which embryonic neural grafts ameliorate deficits in various animal models of CNS disease. Surrounding grafted cells with a biocompatible, impermeable membrane that allows diffusion of small molecules such as O2 and metabolic waste products promotes sustained cell viability but also, isolates the enclosed cells from the physical contact with cells of the host. This approach may reveal the relative importance of interaction between graft-derived molecular diffusion as mediators of graft efficacy. To test the feasibility of this approach, embryonic ventral mesencephalic cells were isolated from E17-19 day old rat embryos cultured in DMEM with 10% FCS either on polystyrene culture plates directly or after being combined, with an artificial extracellular matrix (Matrigel). Semi-permeable capsules containing fetal mesencephalic cells seeded in Matrigel were implanted within the striatum of normal adult male Sprague-dawley rats within three hours of isolation. Animals were sacrificed at two (n=5) and four (n=4) weeks and analyzed histologically. The in vitro culture systems contained non-neuronal and neuronal cells types which many of which stained positively for tyrosine hydroxylase (TH). Viable cells were present in all of the capsules in vivo explanted at two and four weeks which displayed morphological phenotypes similar to those observed in vitro cultures. Some of the cells stained positively for TH indicating that neuronal cells survived within the capsule over the four week period in vivo. Ongoing studies are aimed at optimizing neuronal cell survival and addressing functional efficacy.

393.3

The mouse mutation (Weave) induces a genetic nigrostriatal dopamine (DA) deficiency. Assays of ['H]DA uptake were carried out in vitro in striatal synaptosomal fractions from wild-type mice (+/+), and from the two hemispheres of weave mutant mice (wm/wm) that had received unilateral grafts of mesencephalic cell suspensions to the right side. Amphetamine-induced turning behavior was used to monitor graft survival. Recipient mice were ratied by an average of 22 turns to the left and 7 turns to the right in the non-grafted, and 10 in the transplanted striatum of weave mutants (n=15). Paired comparisons of right vs. left side in the striatum of the weave recipients showed a 38% increase on the grafted side [mean value (R)/L) in general, animals with a strong rotational bias to the left tended to have higher DA uptake values on the right. These findings attest to the functional effects of the grafts. Since DA uptake was measured in entire striatal preparations, the regional extent of DA effects in areas strictly associated with motoric activity may be underestimated. (Supported by USPHS R29-NS29283.)

393.4
Transforming growth factor alpha: A potential role in the efficacy of intrastriatal transplants. S. Loughlin, T. Lee, Y. Ibrahim, D. Teweld and J.H. Fallon. Departments of Anatomy and Neurobiology, University of California Irvine, CA 92717

Intrastriatal transplants of fetal mesencephalon reverse behavioral deficits associated with loss of dopaminergic innervation of the striatum. Transforming growth factor alpha precursor like immunoreactivity (TGFa-LI) is present in a subpopulation of astrocytes (Pallon, et al., 1990) which is increased by fetal transplants (Loughlin, et al., 1989). Transplants of adrenal medulla also ameliorate lesion induced deficits, even when dopaminergic cells do not survive (Bohe, et al., 1989). Adult adrenal medulla transplants are effective in treating striatal TGFa-LI astrocytes (Loughlin, et al., 1992). We therefore hypothesized that TGFa might play a role in the efficacy of transplants. To test this hypothesis, animals were given unilateral 6-OHDA lesions of the dopaminergic projection to the striatum and amelioration of apomorphine (0.2 mg/kg ip)-induced rotation behavior was quantified. One group of animals then received intrastriatal infusions of 200 at TGFa (0.05 ug/ul) in artificial cerebrospinal fluid (CSF) via an Alzet minipump (200 ul) over a two week period. A control group received infusions of CSF. Rotation behavior was quantified and animals were sacrificed. Brains were processed for localization of TGFa-LI. CSF infusions produced a modest increase in endogenous TGFa-LI, while TGFa infusions caused a dramatic increase in TGFa-LI. Rotation behavior was unchanged in animals which received CSF infusions (p>0.4). Infusions of TGFa, however, decreased rotation behavior by 40% (p<0.005). Thus, TGFa may facilitate recovery from 6-OHDA lesions. Whether such recovery reflects regeneration of dopaminergic afferents or other compensatory changes is not known. Since endogenous TGFa-LI is increased by transplants which have been shown to ameliorate lesion induced deficits, it is possible that the efficacious effects of transplants are mediated in part by upregulation of TGFa-LI. This may have important implications for the development of new treatments for Parkinson's Disease. Supported by NS 26761 and the American Parkinson Disease Association SCC.
393.5

SUPERPARAMAGNETIC CONTRAST AGENTS FACILITATE MAGNETIC RESONANCE IMAGING OF NEURAL TRANSPLANTS IN RAT BRAIN IN VIVO. Andrew B. Norman, Stephen R. Thomas, Ronald G. Pratt and Robert B. Norgren. Departments of Psychiatry and Radiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Although transplants of rat fetal striatal tissue can be observed in vivo using magnetic resonance (MR) imaging, the transplanted tissue is approximately isointense with the host brain, making it difficult to distinguish from host tissue. We have used superparamagnetic ferrihydrate particles coupled to wheat germ agglutinin (WGA) to label and to visualize the transplant in vivo. Dissociated rat fetal striatal (E 15-17) was incubated with WGA-ferrihydrate particles for 2-5 min at 37°C and then transplanted unilaterally into rat striatum. Six days and 25 days following transplantation, rats were imaged at 0.14 T using a T2 weighted protocol (TR=500 ms/TE=30 ms). After imaging, the rats were perfused and brain sections stained with cresyl violet or hematoxylin for iron. The labeled transplant was clearly visible on MR images as a feature characterized by very low signal intensity within the host brain. At higher concentrations of WGA-ferrihydrate particles there was also a corona of high signal intensity, representing a susceptibility artifact surrounding the area of low signal intensity. Histologically, the ferrihydrate particles were observed to be mainly restricted to the area of the transplanted tissue and had a patchy distribution. The cells adjacent to the particles appeared to have normal morphology and cytochrome oxidase activity. These studies demonstrate that WGA-ferrihydrate particles remain associated with transplanted cells for at least 3 weeks and provide a means of monitoring the number of surviving transplanted tissue using MR images in vivo. (Supported by NSF Grant BNS9315733)

393.7


Eighteen rhesus monkeys are being evaluated quantitatively and qualitatively as to the effect of on-off transplantations using adrenomedullary/peripheral nerve grafts. The monkeys were trained on one of two different operant behavioral tests, involving quantitative measurement of disability produced by unilateral carotid ligation of MPTP. MPTP produced marked deterioration of performance in the affected arm. None of the operated monkeys demonstrated spontaneous recovery (M=4). Five to nine months after MPTP administration, a transcutaneous intraventricular catheter was placed in the caudate nucleus (M=2) or the surgical equivalent without tissue (M=4). Increased speed of performance on the task, and in some cases return to normal operant behavior, was observed in all acografted monkeys and only one of the surgical controls. The degree of recovery appeared to be directly correlated with the number of surviving cholinergic neurons and inversely correlated with the degree of deficit prior to grafting. Supported by NIMH, ROI NS24340 and NR-00165.

393.9


We have transplanted human embryonic mesencephalic tissue containing dopamine cells into the caudate and putamen of eight patients with advanced Parkinson's disease. Implants were unilateral in caudate and putamen (n=2) or bilateral into the putamen (n=6). Fetal tissue of 7 to 10 weeks of gestation was used. Alternate patients were immunosuppressed with cyclosporine A and prednisone. Results showed that at best, postural stability and walking were restored, hand movement became normal, and "off" episodes were eliminated. Drug doses were reduced as much as 50%. 18-F-F-D-Flurorodopa PET scans have been compatible with continued growth and survival of the transplant for up to 33 months. Both immunosuppressed and non-immunosuppressed patients improved, although improvement of some patients and some patients did not benefit. Fetal tissue implants may offer substantial long term clinical benefit to some patients with advanced Parkinson's disease.

393.10

SELECTIVE EFFECTS OF INTRAHIPPOCAMPAL LOCUS CORRELUS GRAFTS ON THE DEVELOPMENT BUT NOT EXPRESSION OF KINDLED SEIZURES. J. Renzi, Z. Kokaia and D. Lindahl, Reinsorative Neurology Unit, Department of Neurology, University Hospital, S-221 85 Lund, Sweden.

Intrinsically noradrenergic locus coeruleus (LC) neurons strongly suppress the development of seizures in the kindling model of epilepsy. We have previously shown (i) that transplantation of fetal LC tissue to the hippocampus or the amygdala-paradox circumvent is 6-hydroxydopamine (6-OHDA) treated, hyperexcitable rats retards seizure development in hippocampal kindling; (ii) that kindling leads to a long-term decrease in basal hippocampal noradrenaline (NA) release from the intrinsically LC-seizored as monitored by intracerebroal microdialysis. We now report that this decrease in basal NA release can be reversed by LC grafts, but not by situatal tissue, implanted unilaterally into the previously kindled, non-derestricted rat hippocampus. However, the LC grafts had no effect on the spontaneous kindling convulsions despite a substantial noradrenergic overexpression of the host hippocampus around the grafts. Bilateral implantation of fetal LC tissue into the hippocampus of previously 6-OHDA treated, kindled rats resulted in a normal or supernormal noradrenergic fiber density in major parts of the hippocampal formation, had no effect on the severity of fully developed kindling seizures. Finally, implantation of fetal LC tissue into the intact brain, produced pronounced noradrenergic hypersensitivensaton of the hippocampus up to about 1 mm from the grafts, did not influence the subsequent development of hippocampal kindling.

In conclusion, these data show that a graft-derived, noradrenergic hypersensitivation of the hippocampus fails to affect both kindling development and established seizures. Furthermore, LC grafts have no anticonvulsant effect when implanted into the hippocampus of noradrenaline-depleted, kindled rats, in contrast to the powerful antiepileptic effect exerted by the grafts during seizure development. The data thus point to a marked selectivity in the effects of noradrenergic implants in the kindling model of epilepsy.
393.11
TRANSLATION OF HCN-1A INTO RAT BRAIN. B. Morgan, D. Pizzo, K. Werrbach-Perez, L. Hutton, Y. Lu, K.N. Westlund, C. Hultebusch, H.M. Eisenberg*, R. Perez-Polo. University of Texas Medical Branch at Galveston, TX 77555.

The HCN-1A is a human neural cell line that responds to the nerve growth factor (NGF) by fully differentiating and is a potential donor for transplantation into brain regions damaged by disease processes that have caused cell loss. It was developed from a child with megalencephaly. HCN-1A grown in 100% DMEM and 0.5% fetal calf serum with daily medium changes are differentiated in 10 ng/ml NGF, 1 mM forskolin, and 1 mM dibutyryl cAMP (NGF cocktail). We have evaluated the cells using immunocytochemical methods and found the cells to contain GABA, glutamate, VIP, somatostatin, and cholecystokinin-B. Immunoactivity of the cells for synaptophysin is increased with the differentiation of these cells. We have shown that these cells express p75NGFR mRNA using reverse transcription and the polymerase chain reaction (PCR) with primers for p75NGFR. The cells are also stained cytochemically with Me 20.4, an antibody to the human p75NGFR in preliminary experiments 5x10^6 HCN-1A cells were implanted into rat motor cortex. Surviving cells were demonstrated two weeks after transplantation of HCN-1A differentiated with NGF cocktail and/or acetyl-L-carbaryl amine into injured rat brain. Supported by a grant from the Mirt by Foundation and the Institute for Senescence, Pomezia, Italy.

393.13

Embryonic day 17 (E17) neurons transplanted adjacent to photolytically induced pyramidal neuron-deficient cortex (lamina III) of early postnatal mice have revealed preferential migration and pyramidal phenotype within the lesioned zone. These results support involvement of potentially altered expression of environmental cues in guiding grafted neurons toward partial restoration of normal cytoarchitecture. The present experiments assess whether these normally developmentally age-specific cues may similarly guide directed repopulation in selectively lesioned older mice at times when non-specific developmental cues would no longer be expected.

Donor neocortical neurons from E14 or E17 mice prelabeled with combinations of fluorescent nanospheres and [3H] thymidine were transplanted into photolytically lesioned 4 or 6 week old mice (n-14). Photolysis of targeted pyramidal neurons followed unilateral injection of latex nanospheres (230 nL) containing the cytoytic chromophore E, retrograde transport by cingulate projection pyramidal neurons in contralateral lamina III and V, and transectional laser illumination with a 670 nm continuous wave laser. After survival times of 1 to 6 weeks, serial sections were cut and processed for autoradiography, fluorescence, and routine histology. Preliminary results suggest that a subpopulation of transplanted neurons from both donor ages assume pyramidal morphology selectively within the neuron-deficient zone and extend processes. This suggests that E14 neurons "destined" to form deep cortical lamina may be influenced by a selectively altered environment to repopulate superficial laminae after normal development is completed. Supported by HD28478, MR Center grant HD16655, the Alzheimer's Association, and the Rita Allen Foundation.

HUMAN COGNITION: BLOOD FLOW/METABOLISM

394.1

A positron emission tomography (PET) study of auditory processing was conducted using four classes of stimuli (syllables, words, tones, triplets) and two auditory task conditions. For each stimulus class, six different stimuli were presented ten times each. Activation of areas was assessed by defining areas of change on one group of subjects, and attempting to replicate these activations in other groups of subjects.

One set of subjects was instructed to listen passively to binaurally presented stimuli while fixating on a dot centered on a display monitor (passive task). Another set of subjects was trained to detect one of the stimuli within each set and to raise their left index finger whenever they heard the target stimulus, while also maintaining fixation (fixation task).

Activation patterns were assessed in a control condition for all tasks (fixation task). Areas which were more active during performance of the auditory detection task than control task included: primary and surrounding auditory cortex, left prefrontal cortex, and the medial frontal cortex. A set of areas localized bilaterally along the intraparietal sulcus were less active in the detection than control task. In contrast, none of the areas outside of auditory cortex were significantly changed during the passive task.

These results suggest that areas beyond the sensory processing regions are affected by task performance. Frontal regions might reflect processing necessary for activation of task performance, while parietal regions might reflect a reduction due to shifting of attention from the visual fixation task to an active auditory task.

394.2

A PET activation study was performed on 12 normal human subjects to examine areas related to working memory for verbal material. For the active tasks, subjects were serially presented 5 visual words or nonwords beneath a fixation cross prior to the start of the PET scan. Task instructions were to fixate while attempting to remember the items without verbalizing them or making mouth movements (monitored with EMG). Subjects recalled the items aloud when used after the scan. Stimuli used in the active tasks were: 1) categorically-related nouns, 2) unrelated nouns, and 3) nonwords. Two control tasks were used: simple fixation, and a recitation control (subjects silently and slowly repeated the digits '12345' while maintaining fixation).

All active conditions relative to fixation showed increased flow in midline structures and a right hemisphere, and decreased flow at or near Rolandic cortex. The activations in the recitation control differed from those of the activation tasks; only a left Sylvian-insular region was activated in the recitation control as compared to fixation. While there was cooling performance in the real word conditions, only half of the subjects recalled 100% of the nonwords. Activation differences found between good and poor performers included an increased activation of a left premotor area in good performers in contrast to an antememorial visual cortical area in the bad performers. There was a significant interaction between the two areas in the good vs. bad performers.

Several points are suggested in a representation of verbal material requires activation of certain areas (SMA, right prefrontal cortex) across many conditions; 2) inhibition of vocalization in these tasks produces decreases in Rolandic areas; 3) some areas differ with level of performance, suggesting a visual (bad performer) vs. phonological (good performer) strategy difference.
394.3 ACTIVATION OF LEFT POSTERIOR TEMPORAL CORTEX IN A VERBAL RESPONSE SELECTION TASK IS RATE DEPENDENT. ME Rauter*, JA Fiez, TO Vision, SE Peterson, Wash., U. Sch. Med., St. Louis, MO 63110. The cortical area in the left temporopolar boundary has been thought to play a significant role in word comprehension since the original observation of Wernicke in the 19th century. It was surprising, therefore, that event-related activation studies, in which subjects were asked to say aloud an appropriate verb for visually-presented nouns, failed to detect activation in this area of cortex. As a control state, subjects were asked to repeat aloud the visually-presented nouns, and the nouns were presented in both conditions at the rate of 1 per second. Five non-temporal areas showed significant activation: anterior cingulate, left prefrontal cortex and right cerebellum increased; while Sylvian insular cortex decreased bilaterally. A second study, using the same conditions, was performed at a rate of 1 word every 1.5 seconds. At this slower rate, there was a significant activation in the left posterior temporal region (x=9, y=-25, z=6); Taberach et al. (1997) accompanied by the previously noted changes. Several explanations are suggested by these results including: 1) the left posterior temporal region acts as a short-term verbal semantic buffer and is more active because the longer presentation time encourages longer storage times; 2) the region may be active because the slower presentation time allows more active and complete semantic processing before that demanded by the verbal presentation task, per se. Other investigators have also suggested that slower word presentation rates produce greater temporal activation (Marshall et al. Soc. Neurosci. 22, 1627). These data provide information into the role of left posterior temporal cortex in language processing, and also emphasize the crucial nature of paradigm design in the interpretation of imaging studies in the human brain.

394.5 FUNCTIONAL LOCALIZATION OF HUMAN Olfactory cortex with Position Emission Tomography. BJ Zatorre*, M. Jones-Gotman, A.C. Evans and E. Meyer, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B8. The cortical representation of human olfactory processing mechanisms was studied by examining regional cerebral blood flow changes with position emission tomography during olfactory stimulation. Eleven normal right-handed subjects were presented with a series of eight odorsants bilaterally during the activation phase; a baseline condition consisted of inhalation with no odor present. We used the paired-image subtraction procedure, averaging the responses across subjects; difference activated regions were plotted by matched magnetic resonance imaging. The principal result was strong activation (>3.6 in all cases) at the junction of the inferior frontal and temporal lobes bilaterally, corresponding to the prefrontal cortex, and unilaterally, in the right orbitofrontal cortex. The results agree with anatomical and behavioral data implicating these regions in olfactory processing, and indicate a functional asymmetry favoring the right orbital area in olfaction.

394.7 AGE-RELATED CHANGES IN REGIONAL CEREBRAL BLOOD FLOW (rCBF) ACTIVATION DURING VISUAL SELECTIVE ATTENTION. CL Greis*; B. Horwitz, J. France, G. Wagner, SI Rapoport, MB Schapiro, LG Ungerleider, IV Hasby. Lab. of Neurosciences, Nat. Inst. on Aging, Bethesda, MD 20892. Nine young (20 ± 3 yr) and 6 old subjects (67 ± 3 yr) performed visual tasks during position emission tomographic measurements of rCBF using [15O]water: face and location matching without selective attention (NSA), with stimuli differing between tasks, and with selective attention (SA), where stimuli were the same for both tasks but task demands differed. Young subjects showed no change in reaction time in SA vs. NSA, old subjects were slower in both NSA tasks than the young (p<0.001) and showed further slowing during SA for faces (p=0.02). Image data were analyzed using Statistical Parametric Mapping. Neither group showed significant rCBF increase in SA vs. NSA for either face or location matching. However, a significant age by condition interaction was seen for face matching, in which the old group showed a larger SA-NSA difference in rCBF compared to the young (p=0.003) in left hemisphere lingual, fusiform and inferior occipital gyr, and right hemisphere fusiform, superior and mid temporal, and inferior frontal gyri. Location matching, old subjects had a larger SA-NSA rCBF difference than did young subjects (p=0.005) in left hemisphere parahippocampal and superior temporal gyri, and only in hemisphere parahippocampal and fusiform rCBF. Our results indicate that the rCBF differences are task dependent. During SA for faces, old subjects show greater use of ventral occipital areas known to mediate face perception. During SA for location, old subjects show greater use of parahippocampal cortex, an area not previously identified in perception of location, which may indicate the use of memory systems by older subjects to perform spatial attention tasks.

394.4 REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING STORY LISTENING. D. Mason*, S. Dehane, N. Trehury, N. Murrinav, I. Cohen, O. Leverter, G. Salmamon, A. Sirota, J. Meckler, S.H. Joliot, CEA, Saclay, France, and L.S.C.P., C.N.R.S. and E.H.S. Paris, France. We have investigated regional cerebral blood flow (rCBF) changes during continuous speech listening. Healthy subjects listened in a resting state to the French texts read by a native speaker in an fMRI scanner. Each subject had six rCBF measurements using PET and [15O]water, a series of three conditions being replicated twice: rest, listening to a text in a language unknown to the subject and listening to a text in French (first protocol, N = 5); rest, listening to a list of French words, listening to a text in French (second protocol, N = 5). Auditory stimuli were presented binaurally over earphones. rCBF data were acquired with individual magnetic resonance images (MRI) and normalized CBF values within anatomically defined regions of interest were then compared across the three experimental conditions in each protocol (ANOVA). Both left (LST) and right (RST) superior temporal gyr were activated in all conditions of auditory stimulation (p<0.0005). They were the only active areas during listening to stories in 'Banul. A left inferior frontal gyrus activation (LIF, p<0.01) was found during word list listening. Listening to the stories in French in the first protocol activated the left and right temporal poles (LTP, p<0.0001; RTP, p<0.0005), and the left middle temporal gyrus (LMT, p<0.0005). These activations were replicated in the second protocol (LTP, p<0.0001; RTP, p<0.005; LMT, p<0.005). When pooling the two samples for this condition, extra-temporal activations were also found in LIF (p<0.05) and in left l Indochnis area (LIEN, p<0.05). These results indicate that, besides regions devoted to single-word comprehension, story-level processing activates additional areas. Experiments are under way to separate the putative role of syntactic parsing, verbal memory and semantic integration.

394.6 NETWORK MODELS FOR MAPPING COGNITIVE BRAIN FUNCTION USING POSITION EMISSION TOMOGRAPHY (PET) AND REGIONAL CEREBRAL BLOOD FLOW (rCBF). B. Horwitz* and P. Kirshna, Lab. Neurosci., Nat. Inst. on Aging, NIH, Bethesda, MD 20892. Understanding how the brain mediates cognitive behavior has been aided by the use of PET and fMRI to measure rCBF during specific cognitive tasks. However, given that multiple regions often are activated during specific tasks, the complex interrelationships that occur during cognition need to be understood in terms of neural networks. The starting point for such analyses is to use correlations among rCBF in different brain loci to investigate brain interactions (e.g., Horwitz et al., J. Cogn. Neurosci., in press; Friston et al., Proc. R. Soc. London B, 1991). However, the appropriate way to perform such analyses, and to use the results to generate network models to account for the cognitive behavior under study, is not clear. We recently have developed an explicit network model for simulating rCBF/PET data that allows one to examine brain functional interactions (Horwitz, Abstr. Soc. Neurosci. 17, 540, 1991). Here, we use this simulation model to determine some of the conditions under which two different correlational approaches (Horwitz et al., which is within-task design; Friston et al., which is an across-task design) produce similar or divergent results. Because the functional couplings between brain regions are specified in the model, we demonstrate for those cases with divergent results that the within-task correlational method better reflects the underlying functional configuration among the brain regions.

394.8 THE EFFECTS OF CLONIDINE ADMINISTRATION ON REGIONAL CEREBRAL BLOOD FLOW AND COGNITION IN THE ALCOHOLIC KORSakov Syndrome. E. Gundersen, A. Mathis, E.P. Daniell, N. Dougall, C. Murray, G.M. Goodwin, G. Fink*. MRC Brain Metabolism Unit, Royal Edinburgh Hospital, Edinburgh, EH10 5HF, Scotland, UK. McIntyre & Mair (TINS 1990 13, 340-344) reported that administration of the γ-agonist clonidine improved amnesia in the Alcoholic Korsakoff Syndrome (AKS). In an attempt to replicate and extend these findings, eighteen AKS subjects were recruited for a two phase trial. In phase 1, half the subjects received acute infusion of 150μg/kg of clonidine in a single dose, half received an infusion of saline. All subjects underwent functional neuroimaging using Single Photon Emission Computed Tomography (SPECT) before and after 30 minutes following infusion. Clonidine infusion resulted in a highly significant increase in anterior cingulate cortex blood flow and an associated improvement in verbal fluency. Subjects then entered phase 2, a chronic double-blind placebo-controlled cross-over trial of clonidine 0.3mg twice daily for 4 weeks, and maintained placebo for two weeks, incorporating an intervening two week wash-out period. Detailed neuropsychological assessments were carried out at the end of each two week treatment period, including the assessment of anterograde memory, attention, staff ratings of cognitive failures and 'frontal lobe' measures. Clonidine treatment had no significant advantage over placebo on any of the cognitive measures employed. We conclude that acute administration of clonidine activated the thalamo-anterior cingulate system, resulting in an improvement in selective attention in general, and target detection in particular. However, chronic treatment resulted in no cognitive enhancing effect, possibly as a consequence of receptor down-regulation.
394.11

To examine the role of genetic determination in the pattern of cerebral response to cognitive challenges, we used the oxygen-15 water method for measuring regional brain blood flow (rCBF) with PET to study five pairs of healthy monozygotic twins (two female and three male pairs: mean age 31 years, range 19 to 54). rCBF was measured while subjects performed neuropsychological tests including the Wisconsin Card Sorting Test (WCST), which is a sensitive indicator of the integrity of the dorsolateral prefrontal cortex in man, and Raven's Progressive Matrices (RPM), which is another complex abstract reasoning task that may involve more posterior cortical areas. Sensorimotor control tasks were designed to be similar to each neuropsychological task in visual characteristics and response mode (verbal for rCBF and finger movement for the WCST). rCBF values were normalized (i.e. expressed as a pixel-by-pixel basis as a percent of the whole brain mean). The similarity of regional brain function within twin pairs was assessed by determining the correlation between first- and second-born twins for each of a variety of brain regions.

Significant (p < 0.05) correlations were found: for WCS-right inferior frontal gyri (r = 0.98, p = 0.002), right anterior cingulate (r = 0.89, p = 0.04), and left thalamus (r = 0.91, p = 0.03); for the WCS control-none; for RPM-left inferior frontal gyrus (r = 0.89, p = 0.04), right temporal cortex (r = 0.96, p = 0.00) and for RPM control-right inferior frontal gyrus (r = 0.92, p = 0.02). left anterior cingulate (r = 0.97, p < 0.005), and right temporal cortex (r = 0.87, p = 0.05). These data may reflect the differential neural systems subserving the various cognitive operations involved in these tasks and may further suggest a high degree of heritability in cognitively related function in the implicated areas.

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394.12
SEX DIFFERENCES IN CEREBRAL BLOOD FLOW DURING SPEECH, S.Y. Bookheimer*, T.A. Zeffiro, W. Galliard, T.A. Blaxton, and W. Theodore, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

Gender differences in cognitive skills and aphasia patterns have led to the notion that the sexes differ in cortical language organization. To test this hypothesis, 16 adult males and female subjects underwent Positron Emission Tomography to measure cerebral blood flow while reading or naming objects. We found both general and task-specific differences in blood flow patterns. On all tasks, females showed greater activity in the posterior cingulate, while males showed stronger activation in motor areas. Females had greater left mid-occipital and angular gyrus activity during reading, while males showed increased mid-temporal activity. Left hemisphere area 44 (Broca's area) appeared more anterior among female subjects. Both groups showed strong left hemisphere dominance on language tasks. Contrary to some theoretical accounts, there was no evidence for relatively greater bilateral activity in either group.

DEGENERATIVE DISEASE: PARKINSON'S I

395.1

Human striatal extracts added to rat striatal homogenates (RTH) cultures stimulates dopamine (DA) neuron growth and neurite extension. This growth promoting activity (GPA) is elevated in Parkinson's disease (PD) striatal extracts, suggesting a compensatory trophic response to DA neuron loss. Initial findings suggest this activity is soluble and diffusible. Therefore, we postulated that human CSF may also contain GPA. Ventricular CSF from PD and non-PD patients (n = 11 for each group) was compared to normal and <10%CSF fractions using Cortico-ns microscopic cultures. Fractions from each patient were subsequently diluted (1:4) using Heat's Balanced Salt Solution and 50 μl added to freshly plated 1000 cells. Cells were growing in 200 μl defrosted media. 24 hr later, the number of neurons with processes (dependence of GABA) was counted in PD similar to the human tissue culture. CSF from PD patients consistently exhibited significantly higher GPA than non-PD patients as well as E5A treated (E5A: PD = 104, non-PD = 11, p = 0.01). Additionally, post-hoc comparisons revealed a significant difference in GPA between PD and non-PD and between PD and PD-BSA. GPA was only observed in >10%CSF. A >10%CSF sample from one patient with PD was separated into 7 fractions using FPLC and added to RTH cultures. GPA was significantly elevated in two of these fractions. These data suggest that 1) a factor(s) responsible for striatal GPA are also present in the CSF of normal patients, 2) CSF-derived GPA is not contained in an RTH-derived GPA, and 3) factor(s) > 10%CSF and may be amenable to further fractionation and purification. Studies are in progress to identify GPA using the appropriate chromatography and as well as the cellular specificity of CSF-derived GPA. Preliminary observations indicate that the cells that produce GPA are likely dopamine producing neurons of the nigrostriatal system. If confirmed, CSF-derived GPA may be a surrogate measure of nigrostriatal system function.

395.2

Extracts of adult human and rat striatal tissue stimulate the growth of dopaminergic (DA) neurons in primary culture. This growth-promoting activity (GPA) is elevated in patients with Parkinson's disease (PD). We have proposed that the increased GPA in the PD brain represents a compensatory response to the loss of DA neurons which could slow disease progression by stimulating sprouting. However, if striatal GPA is decreased in the aged brain, compensatory increases resulting from DA neuron loss may be inadequate to overcome PD progression. In an effort to determine if GPA varies with age, we evaluated striatal and cerebral extracts from 2, 16, and 24 month old rats (n = 16) on the growth of low cell density (3,500 cells/well), E15.5 rostral mesencephalic, dorsalized, primary cultures grown in defined media. Cells were treated with extracts from 24 month old rats possessed significantly more viable, neuron-specific enolase immunoreactive neurons with processes (263 ±) than cultures incubated with extracts from 24 month old animals (F = 0.06). Moreover, neuron viability in culture was inversely correlated with age (r = -0.69). In contrast, extracts of the cerebellum from these animals possessed very little GPA which was not correlated with age. If DA neurons in the aged brain exhibit decreased GPA activity for continued viability, the age related decline in this GPA may contribute to DA neuron loss. Furthermore, a compensatory increase in GPA brought about by the DA neuron loss of PD may be inadequate to overcome PD progression. If striatal GPA in humans exhibits a similar relationship to age. An age-related decline in striatal-GPA would therefore predispose older patients to PD.
395.3 MPTP INDUCED CHANGES IN STRIATAL D2-LIKE Dopamine receptors: a FINGER PRINT CHANGES IN MEGREX R-NAA. R. D. Tod*, J. Colvin, J. Carl, J. S. Pertelt*, Departments of Psychiatry and Genetics, and Department of Neurology and Neurosurgery, Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110

MPTP induced destruction of dopamine producing cells has been used both as a model for Parkinson's disease and as a denervation agent for studying the role of dopamine receptors. In the present study we have determined the temporal pattern of receptor expression in MPTP treated primates. Baboons were unilaterally lesioned by intracardiac artery injection of MPTP resulting in a hemilateral Parkinsonian syndrome. Striata from injected animals were analyzed for titrated spiperone binding, messenger RNA levels for dopamine D2, D3, and D4 receptors and dopamine content. In both caudate and putamen there was an increase of two to seven-fold of eticlopride-displaceable titrated spiperone membrane binding which reached peak levels in about 5 days and declined to baseline levels by about 2 weeks following MPTP injections. These increases and decreases in binding site number occurred in the presence of 98% reductions in dopamine in the ipsilateral caudate and putamen. Putamen messenger RNA levels were quantified by reverse transcription coupled to polymerase chain reaction amplification using receptor specific oligonucleotides. In contrast to results for D2-like binding sites, there were no observable changes in the amounts of messenger RNAs for D2, D3, or D4 receptors. These studies suggest that MPTP induced changes in striatal D2-like receptors are secondary to translational or post-translational effects on receptor number.

395.5 MODELING CHAOTIC DopAMINERGIC NEURODYNAMICS. J. Sals, S.H. Price, A.D. Will and J.M. Toss*. Neurology and Psychiatry Services, Jerry L. Pettis Veterans Affairs Medical Center, Loma Linda, CA and Dept. of Neurology and Psychiatry, Loma Linda University School of Medicine, Loma Linda, CA 92350

A nonlinear dynamical model of the nigrostriatal dopaminergic system was proposed by King, Barchas and Huberman (PNAS 81:1244, 1984) who demonstrated the model's potential for explaining the rapid fluctuations in movement observed among certain Parkinson's patients following chronic treatment with L-dopa. We have explored the complex dynamics of a modification of this model. The modifications may be equated with biological variables involved in the pathophysiology of Parkinson's disease. We show that by varying one or both of two parameters, χ, representing mean firing rate of nigrostriatal neurons and γ, a variable proportional to postynaptic dopamine D2 receptor density, the system exhibited a wide range of dynamics. When the variable χ, representing the mean firing rate, was decreased we observed the appearance of a novel family of solutions which exhibited a decreasing incidence of chaotic states. Additionally, we observed the chaotic regime breaking up into a collection of discrete groups of firing states. When the variable γ, which is proportional to dopamine D2 receptor density, was decreased we observed a shift in dynamics from chaotic to nonchaotic states. On this basis we predict that premorbid nigrostriatal dopamine D2 receptor denatilization will influence sensitivity to chaotic dynamics and may influence susceptibility to Parkinson's disease and other movement disorders. We believe these findings may also have important implications in light of recent work being done in the area of experimental control of chaotic dynamics.

395.6 ON-OFF EFFECTS OF DIRECT Dopamine agonists in UNILATERAL NIAGRAL RATS. P.B. Silverman*, Dept. of Psychiatry, University of Texas Health Sci. Ctr., Houston, TX 77030

As Parkinson's disease and its treatment progress, the response to pharmacotherapeutic agents becomes increasingly erratic with the development of dyskinesias and "on-off" effects. In the unilaterally 6-hydroxydopamine lesioned rat, with respect to extent of dopamine depletion, a condition of advanced head-parkinsonisms exists. Here lesioned rats were tested repeatedly with selective direct-acting dopamine agonists and their rotational (circling) response recorded. A remarkable degree of real dose fluctuation was seen when the selective D2 agonist, bromocriptine, or one of the selective D1 agonists, SKF 82958 or SKF 72864, was administered a few days in. In consecutive daily sessions responses of individual animals varied from zero to hundreds of rotations per hour. When treated daily for 2 weeks, SKF 72864, some rats showed a pattern of response which appeared to be a 2 to 3 day cycle of response fluctuation. In others, as well as in the group as a whole, no consistent pattern of response fluctuations continued when treatments were spaced at 3 day intervals. This approach may prove useful for the study of "on-off" effects.

Supported by NIDA grant DA062169.


We are using a digitizing tablet based pointing task developed by Gordon and Ghez (CSH Symp Quant Biol 55:837-847, 1990) to examine the effects of sensory information and instructions influence basic task performance in normal middle aged adults and patients with Parkinson's disease. With arms hidden, 9 subjects (6 normals, aged 39 to 62, and 3 patients with Parkinson's disease (PD), Hoh and Yah ages 1 or II, aged 61-72) moved a cursor to 6 distances (2.4 to 26.4 cm) in an oblique right or leftward direction to match targets seen on a computer monitor. Without reaction time constraints, subjects were told to make uncorrected, straight movements. In 6 different session types, accuracy, brief movement time or both were stressed while we provided or withheld continuous feedback of hand position and/or knowledge of results. All subjects (45 sessions to date) reached different distances by scaling both velocity and duration, although velocity had a more consistent effect on performance (ascertained by multiple regression) in every session but one. Not only velocity and duration, but acceleration and time to peak acceleration were scaled to distance. PD patients had trajectory formulation similar to normals, but one patient (Stage II) had prolonged trajectories which further slowed and undershot distant targets if knowledge of results was unavailable. The PD group was also normal in the PD subjects while still revealing evidence of both bradykinesia (slowing) and excessive dependence on visual cues.

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DEGENERATIVE DISEASE: PARKINSON'S I
WEDNESDAY PM

395.9 DEMENTIA IN PARKINSON'S DISEASE: CORTICAL INVOLVEMENT WITH A FRONTAL PREDOMINANCE USING THE IMMUNODETECTION OF ABNORMAL TAU PROTEINS. P. Vermersch 1, A. Delacourte 1, E. Lavoy-Angel 2, 3, J. Hasse 1, Y. Agid 2, 3 U 156 INSERM, 59045 Lille, 2 U 289 INSERM and Neurophysiology Research Unit, 75615 Paris, France.

The dementia, frequently associated with Parkinson's disease (PD), is generally considered to be of the subcortical type but the high frequency of Alzheimer pathology in dopa-treated patients indicates that dementia may be due to coexisting Alzheimer’s Disease (AD). In this study we analyzed the neurochemical changes in the lesioned dopamine pathway of PD patients.

Results: The frequency of immunodetection of the normal tau triplet was statistically higher in the demented subgroups than in the non-demented subgroup of PD in the prefrontal (p < 0.05), the temporal (p < 0.01) and the entorhinal cortex (p < 0.02) but not in occipital and circular cortex. A quantification of normal tau triplet by densitometry showed that, in opposition to the results obtained in AD patients, the intensity was higher in the prefrontal than in the temporal cortex of most of the demented PD patients.

Conclusion: This study: i) gives a biochemical evidence for the presence of AD changes in demented Parkinsonian patients; ii) suggests that lesions of the prefrontal cortex may significantly contribute to the occurrence of cognitive changes at least in some PD patients.


The aim of the study was to determine whether the speed of serial comparisons of sets of 2, 3 or 4 memorized digits with a target digit (Sternberg paradigm) is different in "on" and "off" states of levodopa and related to the levodopa plasma level. 18 parkinsonian patients (age 61.2; 10.1, disease duration 6.8; 3.1 years) were tested in the "on" and the "off" states of levodopa ("offs") and in states of optimal response to oral levodopa ("on"). Central processing time was not different in "on" and "off" (134.12; 129.51±18 sec) and significantly slower in "off" than in 13 age-matched normal controls (82±20 sec, Mann-Whitney U test). Central processing time was not related to the levodopa plasma level and the motor scores (Schwab rank correlation). In 3 patients significant improvement of arousal was observed under levodopa (affect-arousal scale of Bond and Lader) and in parallel marked acceleration of cognition suggesting that levodopa might have an activating effect on cognition in simple patients with deficient arousal in "off" states.

396.1 REFEEDING AFTER STARVATION INCREASES HYPOTHALAMIC NEUROPEPTIDE Y (NPY) MESSENGER RNA. R. Briones-Urbina 1, S.D. George 2, L. Schwartz 1. 1 Depts. of Medicine and Pharmacology, University of Toronto, Toronto, Ont., CANADA M5G 1A8.

NPY is a potent orexigen when administered into the CNS. Physiologically, it appears to be a neural factor regulating feeding behavior. Hypothalamic NPY gene regulation in response to starvation and refeeding was studied in male Sprague Dawley rats to assess a possible role of NPY not only in the regulation of feeding but also in the maintenance of feeding behavior and body weight. Animals weighing 180-200 g at the onset of the study were housed in environmental rooms in 12h dark/light cycles, with free access to water and food, handled and weighed daily. After an adjustment period of 2 weeks, groups of rats were starved for 12, 24, 48 and 72 h with free access to water. Weight losses of 10, 17, 23 and 30% occurred in each group respectively. Hypothalamic NPY mRNA levels were detected by Northern blotting analysis using a 32p labelled oligodeoxynucleotide probe complementary to bases 1632-1669 of the rat NPY gene. Increased NPY mRNA was detected as early as 12h, peaked at 48h and remained elevated until 72h. Groups of rats were then starved for 24 or 48h followed by free access to chow and water for a further 12, 24 and 48h. After 12 and 24h of refeeding following both 24 and 48h of starvation, there was a further increase in NPY mRNA levels coinciding with weight regain up to 80%, but no further increase was noted after 48h refeeding. We report time-dependent further increases in hypothalamic NPY mRNA levels in refeeding following starvation coinciding with the period of enhanced food intake, rapid weight gain and reversal of body weight to prestarvation levels. These results suggest a physiological role for NPY in the initiation and maintenance of feeding behavior and likely in the control of body weight as a reflection of energy balance.


Neuropeptides such as neuropeptide Y (NPY) and galanin may play a role in regulating circannual cycles of feeding in the golden-mantled ground squirrel (Spermophilus undulatus). To investigate this, we analyzed the distribution of NPY and galanin mRNA in ground squirrel brain using in situ hybridization histochemistry, in rats and mice, NPY mRNA was abundantly expressed in the hypothalamic arcuate nucleus and was also present in the cortex, hippocampus and reticular nucleus of the thalamus. Hypothalamic galanin mRNA was concentrated in the arcuate nucleus and the dorsomedial nucleus. Preliminary findings show that seasonal changes in feeding in ground squirrels is related to changes in the hypothalamic expression of neuropeptide mRNA. Thus, in hyperphagic (food intake = 29.8 ± 0.7 g/day; m4) we observed a reduction in NPY and galanin, whereas in a non-hyperphagic (12.1 ± 1.3 g/day; m4) animals, the reduction in food intake was paralleled by a decrease in hypothalamic expression of NPY and galanin, although a larger sample size is necessary to confirm this. Thus, golden-mantled ground squirrels expresses the genes encoding hypothalamic NPY and galanin in a distribution compatible to non-hibernating rodents. Seasonal changes in the expression of these neuropeptides may contribute to circannual cycles of feeding.

INGESTIVE BEHAVIOR: NPY, GALANIN AND INSULIN


Problem-solving ability in Parkinson's disease (PD) patients, elderly control subjects, and young control subjects was studied using the Halstead Category Test. The subjects were 61 PD patients, 40 age-matched elderly controls, and 46 young controls. Errors on the Category Test did not differ significantly (t tests) between the PD group (4.3±1.2), the elderly control group (4.4±1.4), and the young control group (3.9±1.2). The only indication of greater problem-solving difficulty in the PD group was the somewhat greater percentage of PD patients (58%) as compared to elderly controls (29%) who were impaired by ≥ 2 SDs from the young control group mean. However, an equal percentage of PD patients and elderly controls (26%) scored above the mean of the young control group. Our findings do not support previous reports of impaired complex problem-solving ability in PD patients.
396.4 NEUROPEPTIDE Y PROJECTION FROM ARCULATE NUCLEUS (ARC) TO PARVOCELLULAR DIVISION OF PARAVentricULAR NUCLEUS (pPV): SPECIFIC RELATION TO CARBOHYDRATE FEEDING. M. Hnasek-Unial*, B. Beck, Y.S. Hnasek, C. Burlet and S.F. Leibowitz. The Rockefeller Univ. New York, N.Y. 10021 and Faculty de Medicine, INSERM U.308, Nancy, France.

Neuropeptide Y (NPY) injection into the PVN stimulates food intake, specifically of carbohydrate (CARB). The pPV is particularly rich in NPY-containing terminals which originate primarily from the ARC. This study examines: a) the relationship between endogenous NPY and natural preference for CARB; and b) the specific importance of the ARC-pPV NPY projection in this relationship. Sprague-Dawley rats were perimortem hypophysectomized (CARB, protein, and fat), and daily food intake was recorded. Rats were sacrificed 3 ws later, their brains removed, and eight hypothalamic nuclei were microdissected and examined via RIA for endogenous NPY. The results demonstrate that: 1) High CARB eaters, compared to low CARB eaters, have significantly elevated NPY levels specifically in the pPV (p<0.01) but not the magnocellular PVN; in ARC (p<0.01); and in dorsomedial nucleus (DMN; p<0.05). 2) No such relationship was seen for fat or protein intake; 3) Endogenous NPY content is positively correlated with 24 hr CARB intake, in the pPV (r=0.71p<0.001), ARC (r=0.57p<0.001) and DMN (r=0.52p<0.01) only and 3) Endogenous NPY levels in ARC, where NPY cell bodies are concentrated, are positively correlated with NPY levels in the pPV (r=0.54p<0.001) and DMN (r=0.56p<0.001) to which the ARC projects. This demonstrates a close relationship between endogenous NPY, specifically of the ARC-pPV projection, and natural preference for CARB.


Since a large body of evidence shows that increased feeding under natural conditions is enhanced by enhanced NPY release in the PVN (PNAS, 88:1093, 1991), we evaluated the levels and patterns of in vivo and in vitro NPY release from the PVN of STZ-induced diabetic rats displaying hypophagia. Exp 1: NPY levels were measured by RIA in 7 microdissected brain nuclei of rats 18 days after STZ or vehicle treatment. STZ rats exhibited marked hyperglycemia, hyperphagia and elevated NPY levels in 4 hypothalamic sites including the PVN as compared to controls. Exp 2: NPY release from the microdissected PVN and ventromedial nucleus (VMN) of STZ rats was then studied in vitro. Both basal and KCl-induced NPY release were lower in the PVN of STZ-treatment rats than that of control rats. However, NPY release from the VMN of STZ-treated rats was unaffected. Exp 3: NPY release in vivo from the PVN of STZ-treated rats was assessed. Perfusions were conducted in the regions of the PVN and 1 wk later (18-20 days after STZ-treatment) the PVN was perfused via the PPC for 3-4 hours with artificial CSF. Compared to controls, NPY levels in PVN perfusates collected at 10 min intervals from STZ-treated rats were significantly enhanced in association with increased food intake. Cumulatively, these results show that NPY release is augmented in the PVN of STZ-treated rats. Since NPY is a potent, naturally-occurring orexigenic signal, our results support the hypothesis that increased NPY secretion, selectively in the PVN, may be the underlying cause of hypophagia in diabetes. (Supported by UP DSK KG 717 (AS) and NIH DK37275 (P3K & S3K), VA Merit Review (CAS)).


This study examined the relationship between hypothalamic galanin (GAL) levels and macronutrient intake in rats. Albinos (n=50) were maintained ad lib on diets of protein, carbohydrate, and fat. After 3 wk, the rats were sacrificed by decapitation, 10 hypothalamic nuclei and the posterior pituitary (PP) were microdissected, and GAL-IR was measured by RIA. Among the 10 hypothalamic areas examined, only the magnocellular paraventricular nucleus (mPVN) revealed a significant relationship between endogenous GAL and daily nutrient intake. In this region, where dense GAL cell bodies exist, positive correlations were observed between GAL levels and fat ingestion (r = -0.62, p<0.01), 24 hr feeding activity (r = -0.73, p<0.01), fat body weight (r = -0.62, p<0.01), and fat intake during the first 90 min of the feeding cycle (r = -0.68, p<0.01), and body weight gain (r = +0.37, p<0.05). While a small inverse correlation between mPVN GAL and carbohydrate intake (r = -0.41, p<0.05) was also detected, there was no apparent relationship between GAL and either protein intake, total caloric intake or body weight. GAL in the PP was similarly related to fat intake (r = +0.40, p<0.05), as well as to GAL levels in the mPVN (r = +0.40, p<0.05). No such correlations with the other hypothalamic areas were seen. This relationship, between natural fat ingestion and endogenous GAL specifically in the mPVN, agrees with studies of GAL mRNA and also with central injection studies showing the PVN to be the most sensitive site to the selective stimulatory effects of exogenous GAL on fat ingestion.

396.7 GALANIN IN THE CENTRAL NERVOUS SYSTEM OF LEAN AND OBSEZ ZUCKER RATS. Beck*, B. Burlet A. Nicolas, J.P. Burlet C. - INSERM U308 MRCA, 38 rue Capucine, 54000 Nancy (France)

Galanin (GAL), a 29 amino acid peptide, is widely distributed in the central nervous system and especially in the hypothalamus. It strongly stimulates food intake when it is administered in the paraventricular nucleus (PVN). The obese Zucker rat with a well-established hyperphagia is characterized by a general dysregulation of some important neuropeptides involved in the regulation of feeding behavior, such as GAL or CCK, but nothing is known about the central status of galanin in these rats. The aim of this study was therefore to measure GAL in different microdissected brain areas in lean and obese Zucker rats. As an index of the central status may modulate changes in the peptide, it was measured in ad libitum fed rats and in 48-h fasted rats. Bilateral arcuate nucleus (ARC) and paraventricular (PVN) and magnocellular (PVmN) parts of the PVN as well as the median eminence (ME) were microdissected and ionotropically perfused. GAL was measured by a specific radioimmunoassay. The two-way analysis of variance revealed a very significant effect of genotype in the PVN (p<0.001) and in the ME (p<0.02). No variation at all were noted in the ARC or in the PVN. Fastig did not influence GAL concentrations in any areas. GAL concentrations were more double in the ad lib obese rats when compared with controls (p<0.05). On the other hand, in the ME where GAL concentration was about 4-fold greater than in the other areas, there was a 20 to 30 % decrease in GAL concentrations in the obese rats (p<0.05). Opposite variations of GAL were therefore observed between obese and lean rats in two distinct areas. Increased PVN levels might be related to the hyperphagia of the rats but GAL did not behave exactly like NPY, an other orexigenic peptide with an ubiquitous action, so no effect on its levels. Its biological action might therefore be different.

396.3 EFFECTS ON FASTING ON GLUTAMIC ACID CARBOXYLASE (GAD) mRNA LEVELS IN RAT HYPOTHALAMUS. A.J. Spoel*, M.W. Schwartz and D.G. Baskin. Departments of Medicine and Biological Structure, University of Washington and Veteran Administration Medical Center, Seattle, WA 98108.

Evidence indicates that the inhibitory neurotransmitter γ-aminobutyric acid (GABA) plays a role in the central regulation of feeding behavior. Since food deprivation increases hypothalamic GAD activity, we proposed that fasting may influence GAD gene expression in the hypothalamus. To test this hypothesis, we measured the content of mRNA for two GAD isoforms (GAD65 and GAD67) using in situ hybridization. Following 24, 48 or 48 hr of food deprivation, coronal brain sections from male Wistar rats (initially weighing 275-300g) were hybridized with probes complementary to either GAD65 or GAD67 mRNA. Northern blot analysis revealed that these probes recognize separate mRNA transcripts. Film autoradiographs were quantified by computer image analysis (ICAy) in the arcuate nucleus (ARC), dorsomedial nucleus (DMN), and ventromedial nucleus (VMN). The VMN showed an increase in GAD67 mRNA expression while no change was observed in GAD65 mRNA. In fed rats, GAD67 hybridization was highest in DMN (0.177 ± 0.002) and ARC (0.170 ± 0.007) with lower values in VMN (0.118 ± 0.007). Food deprivation for 24 or 48 hrs did not alter levels of GAD65 or GAD67 mRNA in any area. However, we observed significant associations between GAD65 mRNA expression in ARC and plasma insulin (r = -0.61) and glucose (r = 0.54) levels. We conclude that: 1) GAD65 and GAD67 mRNA have identical quantitative distributions in hypothalams; 2) hypothalamic expression of GAD65 and GAD67 genes is not affected by food deprivation; and 3) GAD65 gene expression in ARC may be influenced by changes in circulating levels of insulin and/or glucose.
396.9 INSULIN TRANSPORT FROM PLASMA INTO THE CENTRAL NERVOUS SYSTEM IS SATURABLE IN VIVO. G.D. Baur, D.M. Foster, D. Perci Fr, R.N. Bergman and M.W. Schwartz*. Dept. of Bioengineering and Medicine, Univ. of Washington and Seattle VA Medical Center, 98108, and Dept. of Physiology and Biophysics, Univ. of Southern California, Los Angeles, CA 90033.

Circulating insulin enters the central nervous system (CNS) where it acts as a regulator of neurotransmitter and body weight. We have previously shown that the kinetics of insulin uptake into cerebrospinal fluid (CSF) from plasma can best be explained by passage through an intermediate compartment, hypothesized to be brain interstitial fluid. To determine if an insulin receptor contributes to this uptake, we infused anesthetized dogs (n=9) to 90 min euglycemic intravenous insulin infusions to obtain a wide range of plasma insulin levels (69-5046 μU/mL). Plasma and CSF samples were collected over 8 hr for determination of immunoreactive insulin levels, and the kinetics of CSF insulin uptake were analyzed using a mathematical model with three components (plasma→intermediate compartment→CSF). Frequent sampling during rapid changes of plasma and CSF insulin levels enabled the model to precisely identify rate constants (mean standard deviation): 14% characterizing the uptake of insulin from plasma, through the intermediate compartment and into CSF (k1,k2), and clearance of insulin from both intermediate compartment and CSF (k3). At physiological plasma insulin levels (8.3±μU/mL), k1,k2 was determined to be 11.5±1.0 min⁻¹. However, with increasing plasma levels, k1,k2 decreased progressively, being reduced seven-fold at supraphysiologic levels (5046 μU/mL). The apparent Km of this saturation curve was 500-800 μU/mL (3.5 μM). In contrast, the rates of insulin clearance from both the intermediate compartment and CSF did not vary with plasma insulin (k3=0.011±0.0001 min⁻¹ and k4=0.046±0.002 min⁻¹). We conclude that transport of plasma insulin into CNS is saturable. This mechanism is consistent with insulin binding to blood brain barrier insulin receptors and transcytosis through microvessel endothelial cells.

396.11 IVT INSULIN DECREASES RESPIRATORY QUOTIENT IN RATS. C.R. Park*, M. Chavez, S.C. Woods, Dept. of Psychology, University of Washington, Seattle, WA 98195

Centrally administered insulin decreases food intake and body weight in several species. In some studies the observed decrease in body weight appeared greater than what would be predicted solely on the basis of decreased food intake. It is therefore possible that central insulin affects metabolic rate independently of its behavioral effect. Male Long-Evans rats (n=6) received either insulin or saline vehicle into the third ventricle. Bolus injections (4μL in 4 μL) were given twice, 12 hours apart. Water, but not food was available. Respiratory quotient (RQ) was measured by indirect calorimetry. All animals were exposed to both conditions in a counter-balanced design. IVT insulin resulted in a statistically significant decrease of RQ from 0.79 in the vehicle condition to 0.72 in the insulin condition. This change of RQ was accompanied by a significantly greater weight loss during insulin treatment than during saline treatment. Peripheral injections of insulin at the same dosage and time schedule increased RQ. We conclude that centrally administered insulin affects metabolic activity and appears to do so by increasing the utilization of fat stores. These results further support the hypothesis that central insulin serves as a negative feedback signal in the central nervous system for body adiposity.

396.10 INTRAVENOUS INSULIN INFUSION INCREASES DIETARY FOOD INTAKE AND ENERGY EXPENDITURE IN RATS. A.E. Willing, H.S. Koopmans* and E.K. Walls. Dept. of Medical Physiology, Univ. of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Chronic insulin treatment significantly increases daily food intake, but insulin has previously been found to both increase and decrease energy expenditure (EE). To study the relationship between feeding and EE in intravenous insulin, we used the veno cava of 12 Lewis rats (VC fixed and VC varied). In the VC varied group, daily food intake increased from a Ringer's baseline value of 71.5 ± 1.5 to 93.9 ± 1.3 and 112.2 ± 0.9 kcal/day at the 2 and 3 U/day insulin doses (p < .01). EE increased from a baseline value of 54.2 ± 4.1 kcal/day to 63.0 ± 4.9 kcal (p < .05). The 3 U/day insulin infusion and the rats gained weight rapidly. RQ increased from 0.83 ± 0.03 during baseline to 0.95 ± 0.02 at 2 U (p < .01) and remained constant. In the VC fixed controls, daily food intake increased from 73.5 ± 0.7 to 98.2 ± 1.4 kcal/day at 2 U/day (p < .01) which it remained constant. There was no significant increase in EE, but RQ increased from 0.87 ± 0.02 during baseline to 0.94 ± 0.02 (p < .01) at 2 U/day. Over time, RQ decreased to 0.79 ± 0.03 (p < .01). These results suggest that the observed increases in EE that are often obtained when exogenous insulin is administered are a result of the increased food intake that accompanies the insulin infusion. Insulin administration also shifts substrate utilization from fat to carbohydrate. This shift occurs even when there is little change in daily food intake.

397.1 REGIONAL CEREBRAL BLOOD FLOW CHANGES DURING ACTIVE AND PASSIVE FINGER MOVEMENTS: A PET STUDY. T.A. Zeltzer* and M. Haliett. Medical Neurology, VA Medical Center, 10-9N226, NINDS, National Institutes of Health, Bethesda, MD, 20982.

Studies in subhuman primates have shown that many cortical neurons that modulate their activity in relation to voluntary limb movement also modulate their activity during passive movement of the same body part, suggesting a relatively tight relationship between a movement and its sensory correlate. In order to examine this in man, we used H215O positron emission tomography to record regional cerebral blood flow (rCBF) in 8 subjects during active and passive finger index movements. We studied 2 different tasks. In the active condition, each subject performed 4 finger abduction/adduction movements twice per second. In the passive condition, the index finger was moved with the same rate and range while the subject lay quietly. The control condition. Significant changes in rCBF were detected using co-variance analysis and the t statistic.

We found significant contralateral rCBF increases during both active and passive tasks in primary motor cortex, primary somatosensory cortex, supplementary motor area, cingulate motor area and putamen (p<0.1). In all these areas the rCBF increase was greatest in the active condition. Significant rCBF increases were detected in ipsilateral anterior cerebellar cortex, ipsilateral primary motor cortex, contralateral insula and contralateral superior parietal lobule. In many motor tasks, processing of repetitive movement is characterized by tight spatial coupling between activity related to active and passive movement of the same body part.
397.3

MOTOR SYSTEM CBF RESPONSES DURING FINGER MOVEMENTS ARE RATE INDEPENDENT. Scott T. Gratton, Roger P. Woods, Michael Pickles, and John C. Mazzeotta. U.C.L.A. School of Medicine, Los Angeles, CA 90024.

The goal of this study was to determine the effect of performance rate on the magnitude of regional cerebral blood flow (rCBF) responses in the human brain. Formal subjects performed a visually guided and mentally guided tracking task with their dominant index finger during serial position emission tomography (PET) imaging of rCBF. Target speed was set at 0.8, 2.6, 4.4, 24.6, and 32.8 cm/sec. Tracking at these rates spanned the physical limits of non-blastic finger movements. Significant changes of rCBF were identified using analysis of covariance and the t statistic after stereotactic normalization. Movement (versus no movement) was associated with significant (p<0.001) rCBF responses in the contralateral primary motor cortex, supplementary motor area, premotor cortex, and ipsilateral anterior cingulate. The magnitude of the responses in these areas was constant across tracking rates. No other linear or non-linear differences of rCBF associated with a rate effect could be identified in any other cerebral area. The results are in contrast to previous studies of the visual system that have shown a relationship between photic stimulus rate and the magnitude of rCBF responses in V1. Motor system rCBF responses, representing the integral of local neuronal activity, remain constant across performance rates. These results suggest that the observed longitudinal changes in the magnitude of motor system rCBF responses during skill acquisition and functional recovery are not secondary to a rate effect.

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Using oxygen-15 labeled water and positron emission tomography (PET), we have found bilateral parietal increases in blood flow in normal humans performing both immediate and delayed visually guided and mentally guided reaches to visual targets. These increases are sufficiently robust that they are consistently seen in individual subjects. To determine which components of these tasks are required for parietal responses, we have varied the lateralization of visual input and motor output, dissociated the spatial and temporal information provided by the visual cues, and compared visual to auditory temporal cues. Restriction of targets to a single visual hemifield did not result in lateralization changes, but when subjects maintained central visual fixation and pointed to the targets with one hand, parietal responses were stronger contralaterally. When subjects pointed to locations that they chose at random, a robust contralateral parietal response was still seen. The timing of movements in this task was still cued visually by the disappearance of the central fixation target. Substitution of auditory for visual temporal cues resulted in a decrease in the parietal response contralaterally, but did not eliminate the response as compared to a motorless control state. A contralateral parietal response was even elicited by pointing to randomly chosen locations using auditory temporal cues in darkness with eyes closed. The contralateral parietal lobe plays an important role in movements to visible external targets and in movements to internally generated targets in the absence of any visual input. Because all of the movements were cued either visually or auditorily, we cannot say whether the role of the parietal lobe is more closely related to evaluation of the status of the cue or to the actual initiation of the motor output.

397.5

TASK-SPECIFIC CHANGES OF LOCAL BLOOD FLOW WITHIN THE HUMAN ANTERIOR CINGULATE CORTEX: RELATIONSHIP TO LEVEL OF PERFORMANCE. T. Paul, J. Petrides, A.C. Evans. Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4, Canada.

Overpractised and Reversal versions of Speech Colorator, and Manual tasks were used to study the role of the anterior cingulate cortex (ACC) in higher-order motor control. In two separate PET experiments, cerebral blood flow (CBF) was measured in 18 healthy volunteers, using the O-water-bolus method. For each subject, one "baseline" and six "task" scans were performed. Accuracy and latency of motor responses were also measured. On the basis of performance in the Reversal Speech task, subjects could be classified into two groups: "passed" and "failed". These groups did not differ in performance on the Overpractised Speech task. In both experiments, significant CBF changes within the ACC were obtained only in the reversal minus overPractised speech subtraction (Repl.: both groups, Expt. II: passed group only), but also in the overpractised minus baseline speech subtraction in the "failed" groups. The presence of ACC activation in a well practised speech task represents an atypical pattern of brain activity, the occurrence of which might signal failure in a more challenging task.

397.6

LOCALIZATION OF MOTOR AREAS WITH TRANSCRUTIONAL MAGNETIC STIMULATION AND MAGNETIC RESONANCE IMAGING IN HUMAN SUBJECTS. M. Daffertshofer, M. Syren, H. Henningsen, and M. Zimmermann. Departments of Neurology and Physiology, University of Heidelberg, Germany (Sponsor: EMA).

Transcranial magnetic stimulation (TMS) and magnetic resonance imaging (MRI) were combined to map motor fields of the human cortex. We used focal "eight-sided" coils (Novametrix 2 T) to stimulate the cortex as revealed by recording motor potentials by surface EMG electrodes over several muscles of the upper and lower limbs. Focal areas associated with distinct muscles were labelled on the skull surface with a cream rich in water, and numbers were assigned to these labels. Up to 80 sites of stimulation could be discerned. Subjects were then investigated by MRI (Siemens 2 T), and the labelled motor foci were projected to the image of the cortex.

In all 5 subjects studied so far the localizations of the points of motor excitations were consistent with precentral cortical motor fields associated with the limb muscles as were previously established by invasive electrical cortex stimulation. Thus, TMS enables the non-invasive mapping of motor fields with a rather high resolution. This method is valuable to detect changes in the topography of the motor cortex as might occur in amputees.

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TMS can be used to map motor representations noninvasively, but it has not been possible to correlate these maps with brain anatomy. Ten TMS stimulations were delivered to each of a grid of points 1 cm apart on the left scalp while EMG was recorded and averaged from the right first dorsal interosseous muscle (FDI) in 4 subjects. EMG amplitude maps of the area surrounding FDI were roughly circular with a diameter of 1-2 cm for responses more than 60% of maximum response. In each subject the 3-D coordinates of the grid and about 300 points on the hemisphere were acquired with a magnetic digitizer. A sphere was fitted to the grid to determine the line perpendicular to the scalp at the maximum. The other points were used as a surface for registration with the subject's head surface on MRI. The parameters obtained from the registration were used to map the maxima into the MRI and to compute the intersection of the perpendicular line with each slice. The intersections were within about 1 cm of the precentral gyrus. This technique can also be used to map other types of electrophysiological data into images.

397.8

FAST MODULATION OF HUMAN MOTOR OUTPUTS DURING ISCHEMIC NERVE BLOCK IS MEDIATED BY UNMASKING OF INTRACORTICAL SYNAPTIC CONNECTIONS. I.P. Brasil-Neto, J. Valles-Sole, A. Pacual-Leone, A. Cammarota, E.V. Amassian, M. Hallett and L.G. Cohen*. Human Cortical Physiology Unit, Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892 and Department of Physiology, State University of New York Health Science Center at Brooklyn.

The amplitudes of motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) in muscles proximal to a transiently ischemic limb segment increase for the duration of ischemia, and return to baseline level afterwards.

To determine the level in the central nervous system at which this fast modulation of human motor outputs takes place, we recorded H reflexes, maximal M responses, MEPs to transcerebral electrical stimulation (TES), MEPs to TMS, and MEPs to spinal electrical stimulation (SES) from muscles immediately proximal to an ischemic limb segment.

MEPs induced by TMS were larger during ischemia, whereas those induced by TES or SES were unchanged. Maximal H/M ratios were also unchanged, indicating that alpha-motorneuron excitability to segmental inputs was unaffected by ischemia.

Given the predominantly presynaptic activation of pyramidal tract neurons by TMS, as opposed to a more direct axonal activation by TES and SES, these findings suggest that intracortical unmasking of synaptic connections is the likely mechanism for short-term modulation in the human motor system during ischemic nerve block.
397.1

MOTOR NEURON CORRELATES OF DISHABITUATION AND SENSITIZATION OF THE GILL-WITHDRAWAL REFLEX IN APLYSIA
B.D. Hawkins1, T.E. Cohen1, R. Katsel2, & R. Kalil2. 1Swarthmore College, HAVEN, CT; 2University of Medicine & Dentistry of New Jersey, Newark, NJ.

We have developed a simplified preparation, consisting of the isolated mantle organs of Aplysia, which undergoes habituation, dishabitation, sensitization, and classical conditioning of the gill-withdrawal reflex (Hawkins et al., 1987, 1990). We previously established that the LE siphon sensory neurons contribute to the reflex in this preparation (Cohen et al., 1993). To investigate the role of different motor neurons, we hyperpolarized them and found that LGD1 contributes approximately 70% of the response. We have recorded correlations of habituation, dishabitation, and sensitization in LGD1. There is a decrease in evoked firing of LGD1 during habituation, and an increase 12.5 but not 2.5 min after shock during dishabitation and sensitization. Conversely, there is an increase in spontaneous firing of LGD1 2.5 but not 12.5 min after shock. To further analyze the mechanisms of these effects, we first hyperpolarized LGD1 and measured the area of the evoked complex PSP. As evoked firing, there is a decrease in PSP during habituation, and an increase 12.5 but not 2.5 min after shock during dishabitation and sensitization. We also measured the gill-withdrawal produced by a constant number of spikes in LGD1, and found an increase 2.5 but not 12.5 min after shock. These results suggest that habituation in this preparation is largely due to depression at central synapses, whereas dishabitation and sensitization are due to central and peripheral facilitation with different time courses. 2.5 min after shock there is a large peripheral effect, perhaps due to FIP at the neuromuscular junction, and probably because of competing transient inhibition. 12.5 min after shock there is no peripheral effect and significant central facilitation.

397.10


School, Boston, MA 02115.

Previous research has demonstrated that symmetric brain regions are larger than their asymmetric counterparts and that this difference resulted from a greater number of neurons in the symmetric brain (Galaburda et al., 1989). 

Neuropsychologia 28, 833-860, 1987). In this study, we tested the hypothesis that this relationship between cell number and asymmetry would be consistent for different studies as characterized by increased standard labeling.

Nineteen Wistar rats were sacrificed in adulthood by transection of the saline solution by 4% paraformaldehyde. The brains were post-fixed for 46 hours before being placed in buffered sucrose solution, sectioned coronally at 30 μm, and one series of every tenth section stained for Nissl substance with Thionin.

Adjuvant series were immunohistochemically stained for vasoactive intestinal polypeptide (VIP) or parvalbumin. The somatosensory/atomotor cortices of the right and left hemispheres were parceled on the Nissl-stained sections, their volumes determined, and the number of labeled neurons within these architectonic regions estimated.

Consistent with previous findings, symmetric brain regions are larger than asymmetric brain regions. In addition, there is a greater number of parvalbumin- and VIP-immunoreactive neurons in the larger of the two sides. These studies also appear to be greater cell packing density of parvalbumin-, but not VIP-immunoreactive neurons on the larger of the two sides. These results suggest that there may be asymmetric concentration of neuronal types associated with architectonic asymmetry. Because of the different proportion of parvalbumin-immunoreactive neurons in the larger side, the later may have different functional properties consistent with cerebral dominance.

This work supported by NIH Grant NS27119.

397.11

CORTICAL NEURONAL PROTEIN SYNTHETIC CAPABILITY IN HYDROCEPHALIC AND DECOMPRESSED ANIMALS. C.L. Wolfgang1, J.P. McAllister II1, and J.S. Way1. Dept. of Anatomy and Cell Biology, Temple Univ. School of Medicine, Philadelphia, PA.

The biochemical etiology of neurological deficits observed in hydrocephalic neonates and the utility of surgical decompression remains obscure because of the lack of a sensitive marker of neuronal function. Therefore, the present study quantified cytoplasmic RNA and nucleolar volume in order to assess the protein synthetic capability (PSC) of neurons in the cerebral cortex of hydrocephalic kittens. The effects of decompression via ventriculoperitoneal (VP) shunt placement was analyzed using the same techniques. Hydrocephalus was induced in cats at 10 days of age by intracisternal injection of kaolin. Some hydrocepha-
ilic animals received "early" (7-8 days post-kaolin, moderate ventri-
culomegaly) and "late" (11-14 days post-kaolin, severe ventriculo-
mega) VP shunts; all animals were compared to age-matched controls.

Brain sections were stained with azure B for stoichiometric binding to
cytoplasmic and nucleolar RNA. Cytophotometric analysis revealed a 19-
48% depletion in the PSC from motor, association and visual cortices
of both moderately and severely hydrocephalic animals. After shunting,
PSC returned to control levels only after early treatment; late shunt
produced slight improvement, but PSC remained 25-52% below
control levels. Since the normal function of neurons is dependant on
the PSC, it follows that cerebrocortical neuronal function is adversely
affected by infantile hydrocephaly, and that surgical decompression
may restore function, but only if performed at early stages of the disorder.

397.2

IDENTIFIABLE CNS GILL MOTOR NEURONS OF APLYSIA: FINAL COMMON PATH OR HIDDEN LAYER? J. Leonard1, J. Edstrom1, M. Martinez2, R. Ferrus1, R. Goldmeier2, F. Lukowik3, M.K. Hartfield1 Science Center, Newport, OR 97365; Dept. of Med. Physiol., Univ. of Calgary, and Dept. of Zool. Univ. of Alberta, Edmonton, Canada.

The hypothesis that Aplysia gill-withdrawal behaviors could be
adequately explained by parallel monosynaptic reflex arcs between six
PGV gill motor neurons (PGNs) and the LE sensory cluster
(Kupfermann et al., 1974; review in Kandel 1978) made clear,
failable predictions that have stimulated experimental work for many
years. Results show that the hypothesis is incorrect, both in detail
(LE cells fire after Cohen et al., 1991; other cells active, Zecevic et al., 1989; etc.) and in principle (PGV
necessary for neither behavior nor learning, Petetz et al. 1976;
behavior not reflex, Leonard et al., 1989; behavior not necessarily
 correlated with MN acticity, Colebro & Lukowik, 1988; Leonard
et al., 1991; In press). Further experimental work requires a new
hypothesis consistent with the available data, i.e. that the CNS is
sufficient for these behaviors and that the CNS mediates behavioral
state and the two interact to produce the behaviors of the intact
preparation. We propose that the known CNS GPNs along with a set of
inhibitory GPNs, can vary in efficacy, both individually and in
concert, with learning and behavioral state and that the parallel CNS
pathways act to set the gain of the gill behavior and to phase shift the
int II network. The model suggests that the CNS GPNs are a biological
realization of the hidden layer of a neural network. Supported by MRC
(Canada), AMFHR, NIMH & NSF.

Previous studies indicated that habituation results in a decrease in the number of active neurons responding to the siphon touch and demonstrated the diversity of habituation effect on individual neurons. In the present experiments, a longer habituation training (up to 60 stimuli with 30 sec of inter-stimulus interval) were given. The habituation effects on both the overall neuronal response and the response pattern of individual neurons were examined. The overall neuronal response shows a good correlation with the decrease in gill contraction during habituation. They both have two phases: a rapid decrease at the beginning of habituation followed with a plateau (zero for the gill contraction). The plateau in the overall neuronal response indicates that the system maintains a constant response level (20-30% of control level) even after complete habituation was achieved. Additional analysis will aim at attributing these two phases to individual neurons or groups.

Supported by NIH grant #NS08437.


Two second messenger pathways, mediated by CAMP-dependent protein kinase A (PKA) and by protein kinase C (PKC), are known to contribute to the presynaptic facilitation of the gill withdrawal reflex in Aplysia (Braha et al., 1986; Sacktor and Schwartz, 1986; Sugita et al. 1992). We studied the relative contribution of each of these on STR-induced short term facilitation at nondepolarized, partially depressed (reduced to 40% of their initial level) and highly depressed synapses (reduced to 10% of their initial value) and on the spontaneous release. We used Rp-CAMP, a specific PKA inhibitor, and H7, a general kinase inhibitor that preferentially blocked PKC in intact Aplysia neurons. Our results show that the PKA inhibitor 1) completely prevents the facilitation of nondepolarized, 2) blocks a small component of the facilitation of highly depressed synapses and 3) has no effects on the modulation of spontaneous transmitter release by SHT. By contrast, H7 1) has no effect on the facilitation of nondepolarized synaptic connections, 2) blocks only partially the facilitation of moderately depressed synapses, 3) blocks a large component of the facilitation of highly depressed synapses and 4) blocks completely the enhancement of spontaneous release by SHT. Our results suggest that whereas activation of PKA is sufficient to trigger the facilitation of nondepolarized synapses, activation of both PKA and PKC is required to facilitate depressed synapses and that the contribution of PKC becomes progressively more important as synaptic transmission becomes more depressed.


The synaptic growth that accompanies S-HT-induced long-term facilitation of the sensory-to-motor connection in dissociated cell culture is associated with a down-regulation of cell adhesion molecules (αPCAMS) on the surface membrane of the sensory neuron (Mayford et al., Science, 1992). Down-regulation is achieved by a protein synthesis dependent activation of the endosomal pathway leading to internalization of αPCAMS (Bailey et al., Science, 1992). Unlike classical receptor-mediated endocytosis, the endocytosis of αPCAMS is triggered by the binding of ligand to a heterologous receptor, the S-HT receptor, and consequent internalization of αPCAMS. To explore the mechanisms of this novel form of transmitter receptor endocytosis in sensory motoneurons, we examined the effects of CAMP, a second messenger activated by S-HT, on the distribution of gold-conjugated mAb specific to αPCAMS. We found that a 1.5-h incubation in CAMP + IBMX (10 -5 ) stimulated the action of S-HT. It led to a 50% decrease in gold-labeled complexes on the surface membrane (10.8 ± 1.1) when compared to either untreated cells (22.6 ± 1.8; p < 0.001) or cells bathed in IBMX alone (122 ± 2.4; n = 5; p < 0.01). Accompanying the decrease in surface labeling was a sevenfold increase in the percentage of gold within the cell (31.6 ± 1.4 vs 4.3 ± 0.9; p < 0.001). These findings suggest the endocytic activation triggered by S-HT can be mediated through the CAMP cascade (either alone or in combination with other second messengers) and now allow us to track the steps within the sensory cells whereby S-HT binding to its extracellular receptor leads to the internalization and degradation of αPCAMS.


One characteristic feature of long-term memory to the tail-withdrawal reflex is that it requires both RNA and protein synthesis, accompanied by structural changes. In an attempt to identify the molecules involved in the process, we have cloned and sequenced one of the serotonin (S-HT)-induced proteins in the sensory neurons of the tail-withdrawal reflex of Aplysia. The protein sequence inferred from the cDNA clone demonstrated that it is the light chain of clathrin. 5HT mapping analysis showed that the steady-state level of clathrin light chain mRNA was increased by S-HT, suggesting that S-HT induces clathrin light chain mRNA expression. In addition, Northern blot analysis indicated that the Clathrin light chain is encoded by a single gene. This gene has all the important structural and functional domains of both light chain A and B of mammalian clathrin, suggesting that it may represent the original form from which the vertebrate chains developed. Serotonin increases the number of clathrin-coated vesicles and neurites in Aplysia sensory neurons. These coated vesicles are involved in the internalization of Aplysia cell adhesion molecules and probably contribute to the structural change of sensory neurons during long-term facilitation.


Tail shock-induced sensitization in Aplysia produces spike broadening and increased excitability in tail sensory neurons (SNs). Both of these effects are mimicked by serotonin (S-HT) but can be pharmacologically dissociated: cyproheptadine suppresses the S-HT-induced spike broadening but not increased excitability (Mercer et al., 1991). We report here that these two modal effects of S-HT can also be dissociated by their time course.

We recorded extracellular spike responses in tail SNs taken before sensitization and examined both spike broadening and excitability. A range of S-HT concentrations (0.5μM-5μM) produced significant increases in spike duration and spike broadening with no change in the duration of excitability (3.2 ms ± 0.3 repectively). However, at 9μM S-HT induced spike broadening lasted significantly longer than increased excitability (3.2 ms ± 0.25 ms; p < 0.01) and more. In contrast, the excitatory response was observed only under the 9μM S-HT concentration. This suggests that spike broadening may have a threshold level to produce excitatory action potentials. The time course revealed that both of these effects follow a discrete washout. At the lowest concentrations (0.5μM) only marginal differences were observed. However, at 2μM S-HT-induced spike broadening lasted significantly longer than increased excitability (3.2 ms ± 0.25 ms; p < 0.01). Thus, serotonin-induced spike broadening and excitability were dissociated in two ways: (1) spike broadening was preferential at carew (200μM) present during SHT applications (N=5); SHT increases in excitatory facilitation were blocked (0.55 ± 0.54%); however, increases in excitatory facilitation was still observed (increase = 73.1%, p<0.005). Moreover, a between-group analysis showed that, even though SHT facilitated the release of GABA, SHT-vegetative facilitation was significantly increased (70 ± 5%); in contrast, SHT facilitated the release of GABA, SHT-vegetative facilitation was still observed (increase = 73.1%, p<0.005). Moreover, a between-group analysis showed that, even though SHT facilitated the release of GABA, SHT-vegetative facilitation was significantly increased (70 ± 5%); in contrast, SHT facilitated the release of GABA, SHT-vegetative facilitation was still observed (increase = 73.1%, p<0.005). Moreover, a between-group analysis showed that, even though SHT facilitated the release of GABA, SHT-vegetative facilitation was significantly increased (70 ± 5%); in contrast, SHT facilitated the release of GABA, SHT-vegetative facilitation was still observed (increase = 73.1%, p<0.005). Moreover, a between-group analysis showed that, even though SHT facilitated the release of GABA, SHT-vegetative facilitation was significantly increased (70 ± 5%);
388.9


Protein kinase A (PKA) has been implicated in presynaptic facilitation in Aplysia. Four forms of the catalytic (C) subunit of the holoenzyme (R(C)C) are the products of a single gene: N1A1, N1A2, N2A1, N2A2 (Glabe et al., 1992). The A1 and A2 polypeptide units arise through alternative splicing of exon cassette encoding residues near the active site and exhibit different substrate specificities and affinities for a regulatory (R) subunit (Biochemistry 30, 15246 (1991)). The N1 and N2 forms arise from alternative promoters and contain complementary N termini originating at a point distant from the active site. Thus, the N termini may be involved not in the direct modulation of catalysis but in substrate targeting i.e. the binding of free C subunit of cellular locations in the vicinity of substrates. Evidence for this comes from in vitro phosphorylation of neuronal membranes using recombinant N1A1 or N2A1 subunits. The N1A1 form phosphorylates two polypeptides of ~30 kDa and ~10 kDa significantly more rapidly then does N2A1. In Aplysia neurons, there exist at least five forms of R subunit, four of which are homologs of vertebrate R (Neuron 8, 387 (1992)). We now provide evidence for R1 subunits in Aplysia neurons: (i) high molecular weight R1-binding proteins have been demonstrated by using 32P-labeled bovine filo to probe protein blots; (ii) rapid CAMP-independent phosphorylation of two ~50 kDa CAMP-binding polypeptides is stimulated by N1A1 or N2A1 subunits. By contrast, the recombinant Aplysia R1A1R1 (type I) subunit is not phosphorylated by C in vitro. R1-binding proteins may anchor PKA holoenzyme near selected substrates. (Supported by the NIH).

388.11

HABITUATION OF THE JUMP REFLEX TO OlfACTORY CUES IN NORMAL AND MUTANT DROsophILA. T. Tuyls* and S. Kos, Beckman Neuroscience Laboratory, Cold Spring Harbor NY 11724 and Department of Biology, Brandeis University, Waltham MA 02254

We are interested to know whether the molecular mechanisms of memory formation after nonassociative and associative learning are similar. Since learning through different sensory modalities may involve different molecular mechanisms and since our associative learning assay is based on odor-odor pairings (Tuyls & Quain, 1985, J. Comp. Physiol. A 157: 263), we have developed an assay for nonassociative learning based on olfactory cues. We found that flies would jump up - as if to fly away - when presented with a concentrated, noxious odor stimulus (10% benzaldehyde), as described by McKenna et al. (1989, PNAS 86: 8118). We then semi-autonomously the olfactory jump procedure to deliver 4x presentations of an airborne odor stimulus repeatedly to individual flies. With such a procedure, the flies eventually stopped jumping.

This response decrement in wild-type (normal) flies shows many definitive behavioral properties of vertebrate habituation: More stimulus trials are required for a fly to stop jumping if the interval of time between trials (ITI) is longer. Fewer trials are required for a fly to stop jumping if the odor concentration is lower. After habituating, 80% of flies will jump in response to the odor stimulus if they have been habituated by a novel, strong stimulus (75 s of violet). Analysis of the (associative) learning/memory mutants has revealed that a) acquisition of habituation is slower than normal, spontaneous recovery (memory retention) is faster than normal and dishabitation is normally low in dance mutants and c) acquisition, spontaneous recovery and dishabitation is normal in amnesic and lather mutants. In addition, acquisition of habituation is faster than normal in mutants with abnormal olfaction or locomotion. These results are consistent with the notions that a) the chocolate mutation affects memory, b) the dance mutation affects both memory and sensory or motor processes and c) short-term memory is normal in amnesic and lather.

388.13

MORPHOLOGICAL AND BEHAVIORAL DEVELOPMENT IN THE EMBRYONIC MEDICINAL LEECH. Shirley A. Reynolds, Andrew Baeder, and William B. Kristan, Jr.* U.C.S.D. Biology Dept., 0322, La Jolla, CA 92037

As a preliminary to studying the development of neuronal circuits responsible for different behaviors, we have been studying the normal course of behavioral development in the medicinal leech, Hirudo medicinalis. Most leech behaviors are first seen during embryogenesis. To establish staging criteria which cover the time of behavioral development, we have been tracking the development of several morphological features as well as the development of both spontaneous and mechanically-evoked behavior. We have found that behavioral responses to light mechanical stimulation vary among different body regions. At the anterior and posterior ends, the predominant response to mechanical stimulation is shortening. The predominant response at the midbody region early in development is circumferential indentation, which is replaced by local bending. A single mechanical stimulus will sometimes produce a combination of two simple behaviors, which can occur either sequentially or simultaneously. The percentage of mechanical stimuli which produce a behavior response reaches maximum at about one week after the animal first becomes responsive. Swimming develops as progressive refinements of the early swim-like behavior. In contrast, crawling is a complex behavior resulting from the successive integration of simpler behaviors, namely progressive elongation and contraction, rear sucker action, and front sucker action. Both crawling and swimming appear to be fully developed by the end of embryogenesis when the juvenile leech emerges from the cocoon. This work was supported by an NIH research grant (MH41390) and by Deutscher Forschungsgemeinschaft.

388.10

HABITUATION AND DISHABITUATION OF THE PROLEG WITHDRAWAL REFLEX IN LARVAL MANUCA SEXTA. D.E. Wiel* and J.C. Weeks, Institute of Neuroscience, University of Oregon, Eugene, OR 97403


Habituation was tested by repetitively deflection a single hair while measuring proleg retraction using either video analysis or a force transducer. Interstimulus intervals ranging from 30 s to 10 min produced significant response decrement. Dishabituation in response to a tactile stimulus delivered to the body wall of the habituated segment was also significant. These studies indicate that the proleg withdrawal reflex of the tobacco hornworm is capable of behavioral habituation and dishabitation. Studies of the neural correlates of these behavioral plasticities are underway.

Supported by grants from NIH, NSF and Patricia Roberts Harris Foundation.
399.1

A RETINA SPECIFIC PROTEIN EXPRESSED TRANSIENTLY ON ZEBRAFISH OPTIC NERVES. H. Chang and W. Gilbert*. Dep. of Cell. and Dev. Biology, Harwood Univ., Cambridge, MA 02138

We are interested in identifying molecules involved in neural development and axonal pathfinding in vertebrates. Zebraschiff provides an ideal system because it can be easily studied at early stage and its nervous system is relatively simple. We have generated a monoclonal antibody, 7A11, which recognized an antigen expressed specifically in zebrafish retina. Immunostaining showed that 7A11 Mabs first stains retinal ganglion cells around 38 hours after fertilization, later (3 days) it also stains inner plexiform layer (IPL) and inner nuclear layer (INL), at adult retina it only stains IPL and amacrine cells. Most interestingly, this optic nerve protein during the initial outgrowth of retina ganglion axons at embryonic stage, but no longer expressed at the adult optic nerve. The expression pattern of 7A11 antigen suggested it may involve in the development and guidance of optic nerve. Immunoblots showed that 7A11 Mab stained a single band at 28 KD, following SDS-PAGE of zebrafish retina sample. Studies are in progress to clone the gene for 7A11 antigen and to determine its role in retina development.

399.3

Mab E1 (SG-11) delineates neuronal cell classes in neuronal-glia interaction systems. A. Zimmermann* and A. Sutter. Institute of Zoology, Technical University, Schmittsplatz 3, 61 Darmstadt and Dept. of Immunopharmacology-Neuroimmunology Group, F. Merck, 61 Darmstadt F.R.G.

Axonal growth processes are controlled by neurotrophic factors as well as by neuronal contact. We describe a neural cell surface antigen E1, which appears to be involved in axonal growth regulation.

Mab E1 defines an early antigen of chick embryonic dorsal root ganglia (DRG). In the DRG E1 antigen is exclusively represented on neuronal cells until embryonic day 7 (E7). At later times it also appears on a fraction of DRG neurons. When antigen expression was analyzed in indirect immunofluorescence staining in single cell neuronal-glia cultures of chick DRG or in DRG explant cultures grown in the presence of nerve growth factor (NGF), it became apparent that E1 positive neurons displayed a growth-promoting effect on the DRG neurons. E1 negative neurons grew associated and became encephalized with E1 positive glial cells while E1 positive neurons did not. When Mab E1 was added to neuronal-glia cultures of chick DRG (E8), the NGF induced neurite outgrowth was blocked completely, suggesting a role of E1 antigen in neurite outgrowth which would appear to be glia mediated in this case of the E1 negative neurons. Also in the CNS the Epitope is widely distributed. Here, motoneurons of the spinal cord constitute one example for an E1 negative motoneuronal cell class growing out into the periphery in close association with E1 positive glial cells.

399.5

NEURITE OUTFLOW FROM CULTURED EMBRYONIC MOUSE RETINAL CELLS IS SUBSTRATE DEPENDENT. *C.F. Lapasset and I.M.H. Hankins.

*Univ. Pittsburgh, Pittsburgh, PA and Medical College of Ohio, Toledo, OH.

Is neurite outgrowth from mouse retinal ganglion cells (RGCs) of different development ages substrate-dependent? Previous studies indicate that RGC neurite outgrowth may be influenced by specific substrate molecules, but it is not clear whether the early developing RGCs show a preference for outgrowth on any particular substrate. To address this issue, we have monitored neurite outgrowth in vitro from dissociated E12-16) or early postnatal (P0-6) CD-1 mouse retinal cells on purified substrate molecules (L1, NCAM & laminin). RGCs from fetal retina were identified by staining with an antibody to neurotransmitter specific β-subunit (TuJ1), a specific marker for early differentiating RGCs (Watanabe et al., J Neurosci 22:85). RGCs from neonatal animals identified in vitro after emulsifying in tissue. This ability for given substrates to support neurite outgrowth was also compared with their expression in vivo.

Cultured embryonic cells expressed a variety of morphologies (unipolar, bipolar and multipolar), and all cells were TuJ1*. Neurite outgrowth on L1 was apparent shortly after cell attachment, and was vigorous after 18-24h (many cells with neurites exceeding 500 μm) (indicating a growth rate of about 1mm/day). Few neonatal RGCs survived beyond 24h, although RGCs survived on L1. Outgrowth from embryonic retinal neurons on non-L1 substrates was detectable only a few days later. The majority of RGCs on NCAM or laminin during one- to two-week cultures, elongation was seen. Neurite outgrowth from RGCs on laminin was seen only rarely. In contrast, cortical cells from the same embryos showed vigorous outgrowth on L1 and NCAM substrates. These data show that developing (E12-16) RGCs prefer to grow on L1 substrates; at some point between E16 and birth, RGCs lose their ability to survive and extend neurites on L1.

399.6

A NEW MEMBRANE PROTEIN ON CHICK SENSORY NEURONS. R.G. Perez*, Y.P.L. Yip, W. Halter, and J. Yip. Dept. of Neurobiology and Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

A 140 kD cell surface protein was identified by a monoclonal antibody (1A12) obtained from a mouse immunized with dorsal embryonic chick spinal cords. The protein is present on cultures of neurons in the CNS and PNS, but absent on glial cells and their axons. In spinal cord, the protein is present on a subset of primary afferents that project to the dorsal funiculi (DF). During early development, the 1A12 antigen is found in dorsal root ganglia, sensory components of peripheral nerves, and central projections in the DF. Later in development (E16-hatching), 1A12 ceases to be found in the DF and is present only in the lateral dorsal horn of spinal cord. In the visual system, 1A12 is present in the retina, optic nerve and optic tract in early stages of axon outgrowth. It is not present in the optic fiber layer of the tectum indicating that the expression of the antigen ceases in optic fibers reach this tissue. By E10, the antigen disappears entirely from the visual system indicating that expression of the antigen is developmentally regulated. The restricted spatiotemporal distribution of the antigen suggests that it is important for the development of different projections.

399.4


We have shown that a protease inhibitor of leupeptin analog, acetyl-Leu-Leu-Val-aldehyde (LLaAl) or benzoylcarbonyl-Leu-Leu-Val-aldehyde (ZllAla) induces neuronal differentiation in P12 cells. In an attempt to identify a target molecule(s), Leu-Leu-Val was immobilized and used as a ligand for affinity chromatography.

Proteins of 35, 35, and 160 kD were isolated from the membrane and cytoplasmic fraction of P12 cells. ZllAla had no effect to induce neurite outgrowth in P12 cells, and 35, 35, and 160 kD did not bind to the ZllAla-affinity column. Several lines of evidence suggest that these proteins are clathrin light chains (35kD and 35kD) and clathrin heavy chain (160kD), components of clathrin which is well known for its role in endocytosis. Separation of clathrin into its heavy and light chains showed the clathrin heavy chain had ability to bind to the ZllAla-affinity column directly. Furthermore, ZllAla but not ZllAla enhanced the rate of polymerization of clathrin triskelion to the coat structure. These results suggest that the role of clathrin should be incorporated into a model describing patterns during the initiation of neurite outgrowth.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992

**399.7** MODULATION OF LAMININ'S NEURITE-PROMOTING ACTIVITY. D. Muzi,* Developmental Neuro-Oncology Lab., Departments of Pediatrics and Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610

Purified laminin (LN) promotes neurite outgrowth by regenerating neurons and NGF-treated PC12 cells. Schwann cells secrete LN and other extracellular matrix molecules including proteoglycans (PGs) that bind to LN but do not affect its activity. In contrast, neurite-promoting activity is completely inhibited when LN is complexed with a Schwann cell-derived, high-density, chondroitin sulfate/heparan sulfate PG. The inhibitory PG appears to specifically address LN since it does not substantially inhibit the neuritic response of PC12 cells to other matrix molecules. Initially I observed, that in contrast to purified LN, LN activity in crude extracellular matrix extracts was not affected by the PG. Other LN-binding proteins including entactin and collagen type IV were not protective. In addition, when purified LN was first complexed with the inhibitory PG, inhibition was not reversed by the addition of LN-binding molecules including other PGs. Neurite-promoting activity was, however, restored (de-inhibited) by treating inhibited LN-PG complex with the metalloprotease stromelysin. Apparent, stromelysin selectively abolishes the inhibitory activity allowing LN to express its neurite-promoting activity. This mechanism for de-inhibition may be particularly relevant since PC12 cells secrete stromelysin at the onset of neurite-outgrowth.

These findings suggest that the neurite-promoting activity of LN is modulated by forming complexes with either protective or inhibitory PGs and this modulation is counteracted by protease(s) secreted during neurite outgrowth.

(Supported by Telesio Pharmaceuticals, Inc.)

**399.8** DEGRADATION FRAGMENTS OF L1 ANTIGEN ENHANCE TYROSINE HYDROXYLASE-POSITIVE NEURITE OUTGROWTH IN MESENCEPHALIC CELL CULTURE. M. Pollrat,* J. B. Williams and W. F. Fried, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20020.

The L1 antigen is implicated in axonal elongation during formation of major fiber tracts and promotes neurite outgrowth in cell cultures that during injury of brain tissue, neuronal surface molecules such as L1 antigen are shed, and degradation fragments may therefore be present adjacent to the damage. These L1 fragments might then influence regeneration and injury-induced growth. We have evaluated neurite outgrowth from tyrosine hydroxylase-positive (TH+) E13 mesencephalic neurons grown in vitro on intact mouse L1 antigen and three degradation fragments separated by molecular weight. Mouse MAG, laminin, fibronectin, poly-D-lysine, and total cell raft served as control substrates. L1 antibodies were added to one set of cultures (experimental), and compared to control cultures containing normal rabbit serum. After 3 days in vitro, the cultures were stained using an antibody against TH, and the length of the TH+ neurites was measured by computer-assisted image analysis in a double blind fashion. TH+ neurites were significantly longer when grown on intact L1 antigen, as well as on each of the three degradation fragments, as compared to the control substrates. As compared to control normal rabbit serum, L1 antibody blocked L1 immunoprecipitation and eliminated the neurite-promoting effect of the L1 substrate and of the L1 degradation products. These data suggest that the presence of L1 fragments in vivo might influence regeneration or synaptic restructuring.

**399.9** NCAM-POLYSIALIC ACID AND L1 EXPRESSION ON GROWING AXONS OF ISOLATED NEURONS. W. T. Kim, W. F. Collins*, and A. N. van den Pol.


Although NCAM has been considered to be a homogeneously distributed neuron adhesion molecule with a general role in development, NCAM expression at the cellular level may vary in molecular density and the amount of polysialic acid (PSA), thereby influencing adhesion and axonal growth. To study the relative densities of NCAM and PSA on the different surfaces of single isolated neuronal cells, we used immunogold cytochemistry and digitally-processed backscatter electron imaging in an Amray 1810 scanning electron microscope. Based on the gold particle density per membrane area from thousands of images representing all surface membrane domains of isolated cells, we found NCAM immunoreactivity distributed evenly throughout the plasmalemma, whereas PSA immunoreactivity different cells in the same culture dish varied as much as 222%. PSA, which may reduce the homophilic binding of NCAM, was strongly expressed on axonal growth cones and their filopodia. PSA immunoreactivity was found at higher densities (37% ± 12% S.E.) on axons than on dendrites of the same cell in 6 out of 7 neurons, and at higher densities (35% ± 11%) on the distal axon near the growth cone than on the proximal part of the same axon in all 7 cells. Since NCAM may influence the homophilic binding of the L1 molecule, we undertook parallel examination of L1. The presence of NCAM-PSA and L1 on the growth cone of axons, together with evidence of a strikingly heterogeneous distribution of these molecules in the cellular microenvironment, support the hypothesis that NCAM-PSA and L1 may participate in some aspects of axonal extension and guidance.

**399.10** DIFFERENTIAL MODULATION OF NEURONAL POLARITY BY EXTRACELLULAR MATRIX AND CELL ADHESION MOLECULES. A. Lochter* and M. Schachner, Department of Neuropathology, Swiss Federal Institute of Technology, CH-8093 Zurich, Switzerland.

The effects of extracellular matrix glycoproteins and cell adhesion molecules on neurite growth have been discussed mainly in the context of promotion of neurite initiation and elongation. Recently, however, there is growing evidence for inhibition of neurite growth into a number of different environments and substrates. The aim of these studies was to investigate the effects of extracellular matrix glycoproteins on the neurite growth of rat sympathetic neurons. Neurons were isolated and grown on polylysine-coated coverslips in standard medium containing 5% horse serum. After 2 days in vitro, the cells were processed for immunohistochemistry with antibodies against the cell adhesion molecule L1 and the lectin concanavalin A. The results of these studies suggest that the presence of L1 fragments in vivo might influence regeneration or synaptic restructuring.

**399.11** GROWTH CONE COLLAPSE INDUCED BY MEMBRANE-BOUND MOLECULES MAY BE MEDIATED BY PERTUSSIS TOXIN (PTX)-SENSITIVE GTP-BOUND PROTEINS. T. Kojima,* S. Yamanaka, S. Matsuura, S. Saitou, T. Varanasi, S. A. Stittmier, and M. C. Fishman, Developmental Biology Lab. and Cardiovascular Res. Ct., Massachusetts General Hospital-East, Charlestown, MA 02129.

Inhibitory signals to growth cones are crucial for the regulation of axonal pathfinding. The transduction mechanisms that mediate such "stop signals" are poorly understood. The collapse of growth cones in culture has been used to partially isolate inhibitory substances (Kumar et al., Science 257:702-705, 1992). We have recently demonstrated that G0 is one of the major proteins in growth cone membranes. In the present study, we examined the potential role of G0 protein in growth inhibition, using the growth cone collapse assay. CHAPS-solubilized collapsing activity of chick brain membranes causes growth cone collapse of both DRG and mouse cerebellar neurons, both in vitro and in vivo. The collapsing activity of DRG membranes is abrogated by octylglucoside-induced elimination of DRG growth cones in DRG neurons, and this effect is also inhibited by PTX. These results suggest that some of the membrane-bound inhibitory signals to growth cones may be linked to specific intracellular responses. However, the exact nature of this process is not fully understood and requires further investigation.

**399.12** PURIFICATION OF GROWTH CONE COLLAPSE-INDUCING GLYCOPROTEIN FROM ADULT CHICKEN GREY MATTER. Geoffrey M. W. Cook, Alan R. Johnson, Marina S. Gordon, Scott A. Akker and Roger J. Keynes.*

Department of Anatomy, University of Cambridge, Cambridge, U.K.

Adult avian grey matter contains a glycoprotein fraction that causes growth cone collapse in vitro (Abatr. Soc. Neurosci. 16: 77, 1990). We have also found collapse activity in mammalian central nervous system substrates. The collapse activity is not extracted with purified polyanionic carbohydrate, but is retained by treatment with proteinase K. In this study, we have purified this activity from chicken grey matter. The avian activity is removed by immobilized proteinase K (PNA), and may be related to a somite-derived, PNA-binding glycoprotein that is involved in the generation of a segmented pattern of spinal nerves. As a further test of similarity between brain and somite activities, chick CNS (E7 retinal) neurons have been grafted into the spinal region of E3 chick embryos; like growing spinal nerves, retinal axons avoid posterior half-somite and grow exclusively anterior half-somite. A purification schedule for the isolation of the glycoprotein from chicken (E9) has been devised. Upon filtration, activity is retained with a membrane of 100,000 M.W. cutoff. The macrosolute is then fractionated by chromatography on immobilized reactive dyes in combination with non-denaturating gel electrophoresis. Material electroeluted from a single band, running just behind a BSA marker on a 10% gel, induces growth cone collapse. We hypothesize that this component may inhibit growth of normal and regenerating axon terminals in the adult brain.
399.15
PURIFICATION AND CHARACTERIZATION OF INHIBitory PROTEINS FROM RAT CNS MYELIN. E. Keller*, R. Rubin, C. Bandlow and M.E. Schwab. Brain Research Institute of the University of Zürich, CH-8029 Zürich, Switzerland.

CNS myelin and mature oligodendrocytes are inhibitory for neurite outgrowth. When myelin proteins from rat CNS are separated by SDS-PAGE, inhibitory activity can be eluted from the 35 kD and 250 kD region. Two monoclonal antibodies (IN-1 and IN-2) were raised against the gel-purified inhibitory fractions and were found to neutralize the inhibitory properties of CNS myelin and oligodendrocytes (Carroll and Schwab, 1990). In addition, IN-1 was found to promote regeneration of transected corticospinal and septo-hippocampal axons in young and adult rats (Schneid and Schwab, 1990; Cadelli and Schwab, 1991). In Western blots of rat CNS myelin, IN-1 and IN-2 bind to a partially overlapping set of bands of approx. 35, 45, 55 and >200 kD. These bands appear to be specific for CNS myelin, since they are not found in PNS myelin. Interestingly, and in contrast to several of the major myelin proteins, some of these bands are resistant to extraction with nonionic detergents, and can only be solubilized with SDS. An immunofinity column was prepared by covalently crosslinking the IN-1 antibody to an IgG matrix. After adsorption of SDS-separated, CHAPS-dissolved CNS myelin to the column, part of the inhibitory activity was retained by the column and could be eluted by moderately increasing the salt concentration. This suggests that the activity binds to the column by means of weak interactions. The inhibitory fraction eluted from the column contained several protein bands between 35 kD and 250 kD that could not be identified. Other proteins eluted by lowering the pH did not show inhibitory activity, nor did they crossreact with the IN-1 (mAb).

399.16
ARE INDUCED ACTION POTENTIALS THE TRIGGER FOR GROWTH CONE COLLAPSE IN SYMPATHETIC PREGANGLIONIC NEURONS? C.P. MacCallum, J.J. Walker, R.I. Hanson. Department of Biogy, University of Michigan, Ann Arbor, MI 48109.

When growth cones of chick sympathetic preganglionic neurons (SPNs) contact dorsal root ganglion neurons (DRGs) in vivo, the growth cones exhibit rapid, oscillatory increases in calcium and collapse. One possible mechanism for this pattern of calcium change is that contact with DRGs induces the generation of action potentials in the SPN. The depolarizing phase of the action potential might create a brief, rapid influx of calcium that would oscillate with the frequency of induced action potentials. To test this hypothesis, SPNs were retrogradely labelled with Dil in vivo, dissociated into single cells, and plated at low density on a laminin substrate. After 16 to 32 hours, the culture medium was replaced with a standard recording medium and whole cell current-clamp recordings were made from SPNs. These neurons had stable resting membrane potentials and did not spike spontaneously. Once a stable resting potential was established, a DRG was manipulated onto the SPN growth cone and the SPN membrane voltage was monitored for the occurrence of action potentials. Our major finding was that in recordings made at either room temperature or at 33° to 37°C, we never detected voltage changes from baseline levels during the ten to twenty minutes of contact with a DRG. This failure to observe action potentials was not due to compromised cell integrity, since action potentials could be evoked in all cells studied. These results suggest that the previously observed calcium oscillations were not due to DRG induction of action potentials in the SPN and indicate that other mechanisms for increasing intracellular calcium should be explored.

399.17

CG4 is a permanent cell line of oligodendrocyte-type-2 astrocyte progenitor cells (O-2A) that can be continuously maintained in the proliferative stage or differentiated to oligodendrocytes (OC) (Louis et al., J. Neurosci. Res., 1992, 30:193-204). The availability of the CG4 line offers a unique opportunity to analyze which factors are produced by O-2A progenitors and their derivatives. Here, we report evidence that culture media conditioned by CG4 cells contian(s) i) an activity that inhibits their own proliferation and ii) an activity that modulates the neurite outgrowth of CNS and PNS neurons. The antiproliferative activity is present in the media conditioned by CG4 cells that are expanded as differentiated O-2A progenitors, as well as by CG4 cells induced to differentiate to OC. The inhibition of mitotic activity is readily complete and fully reversible, and is accompanied by the conversion of the CG4 cell phenotype to A2B5+ cell progenitors to A2B5+ A4+ pre-OC. The production of the growth inhibitory activity does not depend on the nature of the mitogen used to propagate the progenitor OC4 cells (medium conditioned by the B104 oligodendroblasts, PDGF or FGF) and similarly, is not dependent on which mitogen is used in the test CG4 proliferation assay. These observations indicate that O-2A cells constitutively secrete and remain responsive to an autocrine antiproliferative factor. Cultures of newborn rat hippocampal neurons and ER chick ciliary ganglion neurons, were used to determine that after conversion to OC, CG4 cells also release into the medium a polyvalent/ laminin-binding neurite-promoting activity for CNS as well as PNS neurons. A preliminary characterization by gel filtration and cation-exchange chromatography, indicates that the neurite-promoting activity resides in a heat-stable, strongly acidic fraction of Mr >50 kD. Supported by NINDS grant NS-16349.
400.1

To study signal transmission and processing in large assemblies of neurons, patterned networks in which connections may be localized or even specified would be advantageous. We have already developed a method for the formation of self-organized neuronal networks in culture (A. Kawana et al; Soc. for Neurosci., 1991). Here, we describe simultaneous multichannel recording of the extracellular electrical activity and intracellular calcium concentration in such simplified neuronal networks.

A silica glass substrate was employed with 16 or 64 transparent electrodes, each of which was connected to the corresponding metal bond. A metal mask with holes at positions corresponding to the substrate wells was used to locate haptocameral or cerebral cortex neurons selectively in each well. We typically monitored optical signals from 4-8 cells. Under low magnification, each optical signal was divided into three types of cells. From the optical signals, we could distinguish three types of cells: (1) a fast signal, (2) a slow signal, and (3) a signal identified as a fast signal. The fast signal reflected action potentials, and the slow signal revealed glutaminergic excitatory postsynaptic potentials. Based on these results, we have constructed maps representing the spatial pattern of the neural response, and we have suggested a possible embryonic origin of the functional organization of the nucleus of solitary tract in the embryonic chick midbrain, supported by The MESC of Japan and The Mitsubishi Foundation.

400.2
OPTICAL MAPPING OF NEURAL RESPONSE PATTERNS TO GLOSAPHYRGEAL NERVE STIMULATION IN THE EMBRYONIC CHICK BRAIN STEM: T. Sakai, Y. Momose-Sato, K. Sato, A. Hirotta and K. Kamino, Department of Physiology, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan.

In an effort to understand the functional organization/architecture of the brain stem, we have used multiple-site optical recording in order to study the action of the glossopharyngeal nerve in the embryonic chick brain stem. The intact and slice preparations including the brain stem and glossopharyngeal nerve were dissected from 7- and 8-day-old chick embryos and stained with the dye. When a brief electrical stimulation was applied to the glossopharyngeal nerve, optical signals were evoked in the brain stem. They were recorded simultaneously from 127 contiguous sites using a 12 x 12 photodiode array. In the evoked optical signals, two components, a fast, phosphenic-like signal and a slow signal, were identified. The fast signal reflected action potentials, and the slow signal revealed glutaminergic excitatory postsynaptic potentials. Based on these results, we have constructed maps representing the spatial pattern of the neural response, and we have suggested a possible embryonic origin of the functional organization of the nucleus of solitary tract in the embryonic chick midbrain, supported by The MESC of Japan and The Mitsubishi Foundation.

400.3

Normal function of the mature visual system requires precise pattern and axonal connections between the retina and the visual nuclei. Development of this preci pattern involves formation of a rough pattern followed by refinement in the pattern. Evidence suggests this refinement process involves communication between the postsynaptic cell and the presynaptic elements. The neurotransmitter nitric oxide (NO) has characteristics that would allow it to participate in this retrograde communication. This study examined the expression of NO in the developing chick tectum to determine whether it might be present in cells upon which retinal axons terminate. Diaphorase histochemistry was used to reveal the presence of nitric oxide synthase (NOS), the enzyme responsible for NO production. The expression of NOS by cells in the developing tectum coincided spatially and temporally with the arrival of retinal axons in the tectum. NOS expression reached a peak in the tectum at the time that refinement of the initial pattern of connections is occurring. Anterograde labeling of retinal axons with WGA/HRP showed that the processes of NOS positive cells in the tectum were coincident with the region of retinal axon termination. Diaphorase histochemistry with anophthalmic chick embryos showed that NOS expression is dependent on the presence of retinal axons. Northern blot analysis using a cDNA probe for NOS from rat brain (Bredt et al., 1991) verified the histochemical results. These results in the developing chick suggest that retinal axons interact with tectal cells that express NOS which is consistent with the possibility that NOS has a role in the refinement of the pattern of retinotectal connections.

400.4

The topographic projection from the retina to the tectum is thought to be formed by two mechanisms. First, a gross topographic map is formed by axon guidance and position cues, and later a refined map is formed by correlated activity in the retina. We have simulated the formation of a topographic retinotectal map by constructing a two-dimensional neural network based on correlated activity. Hebbian modifiable synapses, and adhesive marker gradients. We first show that in a two-dimensional model of the retina and the tectum, two gradients of adhesive markers are necessary to establish the correct orientation of the map in both axes. The model also shows specificity as well as plasticity in the connections when retinal rotation and hemi retinal ablation experiments are simulated.

Recent evidence has shown that temporal retinal fibers are repelled by posterior tectal membranes while nasal retina shows no preference. To test whether a hemi-retinal adhesion difference is enough to generate a topographic map, we constructed a neural network without gradients which specified that the temporal retina is less attracted to the posterior tectum and the dorsal retina is less attracted to the medial tectum while the nasal and ventral retinas show no preference. We show that a topographic map is formed, and under certain conditions, the map can form more rapidly than the map formed using the two dimensional gradient model. Thus the retina and tectum need not be specifically labeled by two gradients. Instead, hemi-retinal and hemi-tectal differences in adhesion or repulsion that separate each surface into quadrants of differential adhesion/repulsion provide enough positional information to allow proper map formation.

400.5

The cholinergic feedback loop in tectum, which mediates a presynaptic augmentation of retinotectal transmitter release, was either removed using the cholinergic neurotrans AF64A or blocked using nicotinic antagonists. Intracranial injection (IC) of AF64A (30-130 nmoles) resulted at one week in the selective elimination of the cholinergic-mediated polysynaptic field potential following retinal nerve stroke with monosynaptic transmission unaffected, and in the loss of virtually all immunostaining for choline acetyltransferase (CHAT). CHAT staining later recovered in some deep cell bodies but not in the fibers of the SFGS, the retinal terminatory layer. IC injection of AF64A at 18 days postcrush, when regenerating retinal fibers are beginning to form synapses, resulted in unsharpened projections when mapped at 60-80 days. There was a robust topographic map but the multiple receptive fields (MRFs) recorded at each tectal point averaged 34° vs 11° in vehicle-injected control preparations. AF64A treatment before nerve crush also blocked sharpening, ruling out a direct effect on retina/greyness/axon or fibre, as AF64A rapidly decomposes.

IC injection of a Bungarotoxin (BoTX) during regeneration also failed to sharpen the MRFs (P< 0.04). Pure BoTX does not, however, subtractably block the polysynaptic component, the backcalchulation of the monosynaptic field potentials. Interestingly, BoTX has been shown in the goldfish retinotectal system to be a competitive antagonist of the NMDA receptor, blocking the polysynaptic component, but did not fully block the backcalchulation of field potentials. We are still determining if and to what extent sharpness is maintained by blocking the cholinergic system. Supported by NEI grant EY-03736.

400.6

The development of topographic maps has been studied in many regions of the CNS. For technical reasons, however, the development of maps of functional synaptic connections of the visual system has been an object of study. We studied this issue using the corticothalamo (CR) system of the cat as a model. CR axons elaborate their terminal arborizations during the first postnatal month, and adult-like topographic distribution of the axons within the red nucleus is observed only after postnatal day 51 (P 51, Yoshida et al., 1990). Here we carried out intracellular recording from rubrospinal neurons of P1-P28 kittens and examined monosynaptic EPSPs evoked from different sites of the sensorimotor cortex, under Nembutal anesthesia. As expected from the anatomical results, most (26/28) rubrospinal cells in kittens aged P13-P28 showed responses indicating the presence of adult-like functional maps: cells antidromically activated only from C1 and those both from C1 and C11 receive input from the forelimb and hindlimb cortical regions, respectively. However, against what is expected from the anatomical results, cells in kittens aged P1-P7 responded to both C1 and C11 (8/10). This suggests that the basic feature of excitatory connectional map in the CR system is established at birth, before the maturation of CR axons and before clear topography of the axons is observable.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
SENEGATION OF SPINOCEREBELLAR MOSSY FIBER TERMINALS IN +/-LC MALIGN MICE. M.W. Vogel* and J. Princic. MD Psychiatric Research Center, Baltimore, MD 21228.

Spinocerebellar mossy fiber afferents segregate during early postnatal development into sagittal columns. Studies of other mouse mutants and x-irradiated rats (Nunes et al., 1988. J. Comp. Neuro. 273:120-136) suggest that Purkinje cells provide cues for the parasagittal sorting of these afferents. We have examined the distribution of spinocerebellar mossy fiber terminals in the +/-LC mutant to determine if mossy fiber terminal segregation is affected by the death of +/-LC Purkinje cells during the early postnatal weeks of development.

The distribution of spinocerebellar mossy fibers in 1 juvenile (P13) and 4 adult (P6-P9) +/-LC mutants and 1 juvenile (P44) and 2 adult wild type mice (P>90) was visualized by injecting 2% WGA-HRP into the anterior lumbar region of the spinal cord (T13-L2). Cerebella were subsequently processed for TMB histochemistry.

Although the granule cell layer is reduced in size in +/-LC mutants due to increased granule cell death, the distribution of spinocerebellar mossy fiber terminals is similar to that seen in wild type mice. Lumbar mossy fiber terminals predominate in anterior and posterior vermis. While mossy fiber terminal columns are present, they are less distinct than in the wild type. In some areas of the granule cell layer there are clear gaps between regions of dense terminal fields. In other areas, terminal fields are separated only by regions with less dense staining. The poorly defined columns in the +/-LC mutant may be explained either by a failure to form well segregated columns due to +/-LC Purkinje cell death, or by the merging of well formed columns subsequent to granule cell death. (Supported by NARSAD and a NIH Shannon Award)


Part of the habenular projection to the interpeduncular nucleus (IPN) is cholinergic and therefore may be subject to regulation by NGF internalized via membrane bound NGF receptors. Immunocytochemical studies indicate that cholinergic habenular neurons innervate their target subnuclei in the IPN in the second postnatal week, the only period during which lesion induced sprouting can be demonstrated. This suggests that either NGF production or NGF receptors might be developmentally regulated in this system. We therefore used immunohistochemistry to examine the distribution and expression of low affinity NGF receptors (antibody 192, a gift from Dr. Eugene Johnson) during the development of the IPN in the rat. At postnatal day 4, NGF receptor staining is present on axons in the pathway from the habenula that innervates the IPN but is absent in the IPN. By 10 days, recognition of immunoreactivity appears in the intermediate subnuclei of the IPN, the subnucleus receiving densest cholinergic innervation from the habenula. This staining reaches its maximum by 14 days. Thereafter, the receptor staining decreases and is no longer detected by day 28. These observations indicate that habenular axons express the low affinity NGF receptor transiently during the time that they innervate their targets. Supported by NIH Grants NS16556 (MM) and NS28856 (FH).


Mature vertebrates have specific synaptic connections between muscle afferent fibers and motorneurons that innervate limb muscles. Motorneurons receive monosynaptic excitatory inputs from homonymous and synergistic muscles, and polysynaptic inhibitory inputs from antagonistic muscles. To determine whether specific sensory-motorneuron contacts are established during early synaptogenesis in the mammalian spinal cord, we have studied the pattern of these synaptic connections in embryonic (E20-22) and newborn rats (P0-4). Nine hindlimb nerves were stimulated and the evoked potentials were recorded intracellularly from motorneurons of isolated spinal cords. Monosynaptic potentials were distinguished from polysynaptic potentials by their shorter latency and resistance to high-frequency stimulation. Demyelinating CFSRs reversed at 50mV membrane potential. In the embryonic spinal cord, 20% of motorneurons received monosynaptic excitatory inputs from antagonistic muscles. These inappropriate connections persisted until at least 3-4 days after birth, suggesting that some reorganization of synaptic connectivity occurs during postnatal development. Supported by RCDA (NS01314) and NS23088.
401.3

TRKA; AN ALTERNATIVELY SPliced FORM OF TRKA EXPRESSED BY NEURONS. P.A. Basker, C. Lomen-Hoerth, S.O. Meynin, E.M. Gensch, E. M. Shooter, Department of Neurobiology, Stanford University School of Medicine, Stanford, California. 94305.

The product of the Trk proto-oncogene has been identified as a signal-transduction receptor for nerve growth factor. This tyrosine kinase receptor was originally cloned from human K562 cells, a human leukemic cell line. We have identified a variant of the TrkA receptor, TrkAn, which differs from originally cloned form of TrkA by virtue of a small insertion within the extracellular domain. Both forms of Trk receptors are found within rats and humans and the amino-terminal domain of the inserted region is conserved between species. The inserted domain is encoded by a distinct exon whose presence appears to be regulated by tissue-specific alternative splicing. Several nonsense mutations contain both forms of TrkA mRNA. However, only TrkAn is expressed within CNS and PNS neurons of rat and humans.

Rat TrkA and TrkAn have been expressed in Cos cells and shown to specifically bind NGF and display slow dissociation kinetics and tryptophan sensitivity characteristic of the slow NGF receptor. Although the functional role subserved by these distinct forms of the TrkA protein remain unknown, the evolutionary conservation of the inserted domain across species together with the tissue-specific expression of the alternative transcripts indicates some functional role.

401.4


In the retina of a different animal species including non-human primates a significant number of ganglion cells (RGCs) have been reported to express the low affinity NGF receptor (p75-NGFR). The finding that the axonality of the adult retina can be rescued from degeneration by intracocular administrations of NGF led to the hypothesis that RGCs represent a neuronal population of the central nervous system sensitive to the action of NGF. Since the biological activity of NGF is mediated by high affinity membrane-bound receptor, we have studied the immunochemical distribution of both the trk proto-oncogene product p140, which likely corresponds to the high affinity NGFR, and the p75-NGFR, in transverse sections of human retina, to determine if the human retina p75-NGFR immunoreactivity is restricted to Muller cells while a high number of neurons in the GC layer is immunopositive for the p140-trk. On the basis of morphological criteria at least part of these neurons can be classified as RGCs. Conversely, in the rat retina a sub-population of RGCs and Muller cells are immunoreactive for both antigens. Binding studies and affinity cross linking/immunoprecipitation experiments performed in purified in vivo preparations of RGCs from neonatal rat retina confirm the presence of the high affinity NGF receptor. These results suggest that RGCs in humans could be sensitive to the action of NGF and raise the possibility that NGF, or a closely related molecule, could represent a new therapeutic approach for the treatment of retinal neurodegenerative pathology.

401.5

NGF GENE EXPRESSION IN MOUSE AND MAN: ROLE OF RETINOIC ACID RECEPTORS(RARs). C. Jiang*, M. Cartwright and G. Heinrich, Biomedical Medicine, Boston University Hospital, Boston, MA 02118.

Retinoic Acid(RA) induces NGF mRNA in mouse L cells and high and low RA concentrations in NGF binding in L cells and high RA concentrations in NGF binding corresponding to embryonic sympathetic neurons, suggesting an important role in coordinating induction of neuronal NGF dependence, responsiveness to NGF and NGF production region. We have previously cloned the mouse, rat and recently the human NGF gene promoter regions. An intrinsic AP-1 element is conserved in the NGF promoter region of all species, but an upstream AP-1 consensus sequence is human specific. Gel shift analysis showed that the RAR-alpha can bind to the upstream AP-1 consensus sequence in the human NGF but not to the conserved intrinsic AP-1 elements in either human or mouse NGF promoters. Furthermore, the RAR-alpha suppressed binding of AP-1 complex to all AP-1 sites. Several RA response elements(RARE) like motifs are found within 1000bp upstream of mouse and human NGF promoters suggesting direct binding of RARs and transcriptional regulation of NGF by RA in vivo. We are analyzing the differential binding of additional RARs(beta and gamma) to mouse and human NGF promoters and their functional significance, also beginning to address the relationship between the structurally and functionally defined RAREs and the AP-1 consensus sequences.

401.6

NGF INDUCES C-FOS IN THE BASAL FOREBRANNEURS EXPRESSING NGF RECEPTOR (NGFR); A DOUBLE IMMUNOCYTOCHEMICAL STUDY IN THE RAT. Z. C. Papež, M. Fusco, M.G. Núñez* and M. Bentivoglio, Institute of Anatomy, University of Verona; "Fidia" Research Laboratories, Abano Terme, Padova, Italy. 30120.

In order to further elucidate the role of NGF in the brain we investigated the expression of C-Fos, a proto- oncogene product, in brain structures particularly vulnerable to NGF. Mice were injected with NGF (20 µg in 10 µl) into the interpeduncular nucleus, a site known to express NGF in the basal forebrain. The lamination of C-Fos immunoreactivity was assessed in several brain structures of the basal forebrain. Numerous double-labeled neurons were observed, and the latter were slightly more numerous on the side ipsilateral to NGF administration. After the injection of a higher concentration of NGF (20 µg in 10 µl) a stronger response was observed, and the neurons were more numerous. The data suggest that NGF exerts a direct and specific activation of the basal forebrain neurons which express NGFR.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
401.7 LAR TYROSINE PHOSPHATASE RECEPTOR: EXPRESSION IN THE MAMMALIAN NERVOUS SYSTEM. F. M. Longo1,2 and J. M. Le Beau3.

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The Leukocyte Common Antigen-Related (LAR) gene encodes a transmembrane receptor with extracellular domain sequence similarity to N-CAM. Tyrosine phosphatase receptor activity is associated with the growth inhibition of female cell lines transfected with the LAR cDNA and expressing LAR. In situ hybridization of low affinity NGF receptor mice showed that LAR expression was restricted to the nervous system and was not detectable in adult skeletal muscle.

401.9 DISTRIBUTION AND REGULATION OF MEMBERS OF THE TRK FAMILY IN THE RAT BRAIN. I.P. Metello1,2,3, P. Enard1,2, J. Bengzon4, M. Jakob1, Z. Kolay1,2, B. Daras1, O. Lindvall1, and H. Persson4.

Laboratory of Molecular Neuroendocrinology, Karolinska Institute, S-104 01 Stockholm, 2Reproductive Neuroendocrinology Unit, University Hospital, S-221 85 Lund, 3UAB CNRS 1200-Laboratoire d’Histologie-Embryologie, Universite de Bordeaux II, F-33405 Bordeaux

Tyrosine protein kinases (TPK-1 and TPK-2) are signal-transducing receptors for the neuropeptides Nerve Growth Factor, Brain-Derived Nerve Growth Factor, neurophin-3 and neurophin-4. We have identified cDNA fragments encoding a part of trk-A and trk-B that are respectively and characteristically a full-length cDNA clone encoding rat trk-A. Cells expressing mRNAs for the different members of the trk family were identified in the rat central nervous system by in situ hybridization using oligonucleotide probes designed from the isolated cDNA sequences. The expression of trk-A mRNA was found to be restricted to neurons of the basal forebrain, caudate putamen with few magnocellular neurons of the subiculum. In contrast, cells expressing trk-B and trk-C mRNAs were widely distributed in many areas of the brain, including olfactory formations, neocortex, thalamic and hypothalamic nuclei, brainstem nuclei, cerebellum and spinal cord motorneur. Comparison between our data and previous analyses of cells expressing mRNAs for neurophin receptors shows that different modes of action and different combinations of receptor mediate biological responses to neurophins in the adult rat brain. Moreover, seizures induced by hippocampal kindling lead to a rapid and transient increase of mRNA in the hippocampus for trk-A, a function receptor for BDNF. No change was seen in mRNAs for trk-B or trk-C, counterparts of the high-affinity NGF or NT-3 receptors, respectively. The increase of trk-A mRNA was blocked by the AMPA receptor antagonist NBQX. The transient increase of trk-A mRNA showed the same time course and distribution as previously reported increase of BDNF mRNA. This suggests that BDNF and its receptor could play a local role within the hippocampus in kindling-associated neural plasticity.


Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591

The neurotrophins, NGF, brain-derived neurotrophic factor (BDNF) and neurophin-3 (NT-3), appear to bind with similar affinities to the LNGFR, but with markedly different affinities to the trk family of tyrosine kinase receptors. NGF binds to the product of the trk proto-oncogene (trkA), while BDNF and NT-3 are ligands for the related trkB and trkC receptors. While the relative contributions of the trks and the LNGFR to binding affinity and specificity and to signal transduction are now being elucidated in vitro, little is known regarding the co-distribution of LNGFR and trk receptors in vivo. We have examined the relative distribution of cells expressing these different neurotrophin receptors in the adult rat brain using in situ hybridization and immunohistochemistry. LNGFR message and protein are present in restricted subsets of neural and non-neural elements. trkA mRNA appears to be expressed predominantly if not exclusively in neurons, and shows a close correspondence in its neuronal distribution to that of LNGFR. However, non-correspondence of expression of trkB mRNA and LNGFR is apparent in some brain areas. In agreement with retrograde transport and binding studies, cells expressing trkB mRNA are much more numerous and widely distributed than those which express either trkA or LNGFR. Most neurons which express trkB did not contain detectable levels of LNGFR mRNA or protein.

401.11 DIRECTION OF NFG SIGNALLING PATHWAYS BY PERTURBATIONS OF LOW-AFFINITY RECEPTOR (gp75NGFR) INTERACTIONS. S.M. Dunfield1, L.A. Murphy2, R.A. Betteau1, A. Biopolis3, Queen’s University, Kingston, Ontario, Canada K7L 3N6, and 4University of Alberta, Calgary, Alberta, Canada, T2N 2T2.

Growth factors share many signalling events but different biological effects indicate that signalling routes diverge. NGF signal transduction selectivity likely begins at the receptor level because this growth factor interacts with two receptors. To address this suggestion, a peptide (RP3) identical to a cytoplasmic domain of gp75NGFR with predicted amphipathic properties (Myers et al., Soc. Neurosci. Abstr. 17, 1991) was covalently linked to a peripheral plasma target vector. In these NGF-mediated neuronal cell growth in a time frame consistent with its uptake into cells. The cDNA is used in a coexpression system of BDNF and the low-affinity NGF receptor immunoprecipitates. The increase of trk-A mRNA was measured in a number of different brain areas of an adult rat by in situ hybridization. In addition, the above protocols were used to analyze the distribution of LNGFR in vivo. In agreement with previous studies, cells expressing trkB mRNA are much more numerous and widely distributed than those which express either trkA or LNGFR. Most neurons which express trkB did not contain detectable levels of LNGFR mRNA or protein.

401.12 OVEREXPRESSION OF THE CYTOSOLIC DOMAIN OF p75NGFR RESULTS IN DOWN-REGULATION OF p75NGFR EXPRESSION. M. Benedetti1, I. Manni2, and M.V. Cecchini2.

Istituto di Neurobiologia, CNR, Rome, Italy (1997); Dept. of Cell Biology & Anatomy, Cornell University Medical College, New York, NY 10021

The low affinity p75NGFR receptor participates in the formation of high affinity NGF binding sites with the product of the trk gene-coding region. In the formation of NGF signal transduction pathways, a mutant p75NGFR gene was engineered, comprising the transmembrane and cytoplasmic domain of p75NGFR, an N-terminal signal peptide and a myc tagging epitope. This truncated receptor lacked the ligand binding domain. When stably transfected in PC12 cells, neuronal differentiated clones displayed altered responses to NGF. Most notably, in cells expressing this construct, trk tyrosine kinase activity was markedly lowered. This suggests that NGF binding sites, as well as the products of the various NGFR genes, are present in different NGF binding sites. In addition, NGF binding sites, as well as the products of the various NGFR genes, are present in different NGF binding sites. In addition, NGF binding sites, as well as the products of the various NGFR genes, are present in different NGF binding sites. In addition, NGF binding sites, as well as the products of the various NGFR genes, are present in different NGF binding sites.
401.13 EVIDENCE FOR KINASELESS AND ALTERNATE 5' TERMINAL FORMS OF TRKB AND TRKC IN CHICK. A.S. Ganem,* T. H. Large, Department of Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

The 5' flanking region of tyrosine kinase receptors mediate neurotrophin-induced responses such as proliferation, survival, and process outgrowth. Alternative splicing is one potential mechanism for generating diversity at the level of ligand binding and/or signal transduction. To identify possible splice variants and as a prelude to investigating the role of 5' trk receptors in the developing chick visual system, we have isolated cDNA clones of chick trk (trkB and trkC). An 1133 bp chicken brain cDNA library, screened initially with a mouse trkB cDNA probe, yielded a total of 22 trkB and 16 ch1c transcripts. From a 5' cDNA map and amino acid alignment of a full-length chtrkB clone with mouse trkB identifies conserved domains that are likely to be functionally important. Within the extracellular domain, the second immunoglobulin-like domain is strongly conserved (81%). The trkB juxtamembrane domain (between the transmembrane and kinase domains) is also highly conserved (98%) and may be the principal regulator of kinase activity and/or specificity, especially given the small size of the C-terminus (15 aa) and kinase insert (11 aa) domains.

Alternate 5' and 3' splice variants of chtrkB have also been identified. Similar to those reported for rat and mouse, a kinaseless chtrkB clone is truncated just after the transmembrane domain and the 11 alternatively spliced C-terminal amino acids are completely conserved. In alternate 5' terminal clones (chtrkB-Δ5'), the start AUG, signal sequence and first cysteine cluster are replaced with a sequence that contains an upstream stop signal. This sequence is also found in the 5' untranslated region of the full length clone and is flanked by a spliced donor site, indicating chtrkB-Δ5' results from the splicing of 5' untranslated sequence into the extracellular domain.

We have also identified full length, kinaseless, and alternate 5' terminal clones of chtrkC. As with chtrkB, chtrkC-Δ5' clones replace the start ATG, signal sequence, and first cysteine cluster with a novel sequence encoding an upstream, in-frame stop. Alternate 5' transcripts lacking a start ATG may represent an unusual mode of regulating expression. Alternatively, translation may be initiated at a downstream ATG or non-ATG codon, resulting in a trkB receptor with a truncated extracellular domain.

401.15 DORSAL ROOT GANGLION NEURONS EXPRESSING TRK ARE SELECTIVELY SENSITIVE TO NGF DEPRIVATION IN UTERO. S.L. Carroll, K. Ruiz, S.E. Frese, J. Milbrandt and W.D. Snyder*, Departments of Pathology, Laboratory Medicine, and Neurology, Washington University School of Medicine in St. Louis, Mo. 63110.

In utero immortalization of the neurotrophin-receptor nerve growth factor (NGF) receptors in the death of most, but not all, mammalian dorsal root ganglion (DRG) neurons. The recent identification of trkB, trkC, and trkA as the putative high-affinity receptors for NGF, brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT3), respectively, has allowed an examination of whether their expression by DRG neurons correlates with differential sensitivity to in utero deprivation of NGF.

NGF deprivation in fetal rats was produced by injecting a specific, high affinity anti-NGF antibody into embryonic rats in utero (Ruit et al. Neurobytes 8:31, 1992). After 4 hr, the rats were perfused in situ and the DRG sections from control and experimental animals with proks for trk, trkB, trkC, and p75NGFR, the low affinity NGF receptor. We have found that more than 90% of DRG neurons expressing trk are lost when deprived of NGF in utero. In contrast, most, if not all, NGF-expressing trkB and trkC cells were preserved in this treatment. The low affinity NGF receptor, p75NGFR, is expressed in both NGF deprivation-resistant and -sensitive neurons.

These experiments show that DRG neurons expressing trk require NGF for survival and that DRG neurons which do not require NGF express the high affinity receptor for another neurotrophin. Furthermore, they provide evidence that trk, and not p75NGFR, is the primary effector of NGF action in vivo.

401.17 MAST CELLS EXPRESS TRK BUT NOT LOW AFFINITY NERVE GROWTH FACTOR RECEPTOR FACTOR RECEPTOR. J.C. Pryor, K. Horj Sunaj, and E.M. Johnson, Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Conn. 06511.

The physiological response to nerve growth factor (NGF) is best understood in sympathetic and sensory neurons and PC12 cells. Rat peritoneal mast cells (RPMC) release histamine in response to NGF when lysophosphatidylserine is present. We have determined the expression of trk in the RPMC and trk in a mast cell line to understand the receptor(s) mediating the physiological response to NGF in these cells.

Total RNA was isolated from RPMC, non-mast peritoneal cells (non-RPMC) and PC12 cells. Northern blots were done with probes to trk and LANGFR. These blots showed a perfect signal at the appropriate size in mast cells and PC12 cells, but a very low signal in the non-RPMC fraction. A strong message was detected for LANGFR in PC12 cells, but no signal was detected in mast cells or non-RPMC. RNA from mast cells, PC12 cells, and superior cervical ganglion sympathetic nerve culture (SCG) were reverse transcribed and PCR was performed using oligonucleotides specific for trk and LANGFR. Message for trk was stronger in the mast cell fraction than in PC12 cells; the strengthens of the two bands were detected in PC12 cells and SCG cultures, but none was seen in mast or non-RPMC fractions. Radioiodinated NGF was cross-linked to mast cells and PC12 cells; the same results were obtained in immunoprecipitated with polyclonal anti-NGF or monclonal anti-LANGFR. Bands consistent with LANGFR were detected with PC12 cells, but none were detected in mast cell cultures. Our results strongly suggest that mast cells have only one NGF receptor species, trk, yet are capable of responding to NGF with a physiologic response. This immunoreactivity appears to require dimerization between the trk proten- oncogene and LANGFR in mast cells. (Supported by NIH grants NS34670 and NINDS 5 T32 N012025-10, and the Sumanillo Chemical Company.)
401.16 EVIDENCE FOR THE EXPRESSION OF FUNCTIONAL LOW AFFINITY AND txa NGF RECEPTORS IN CULTURED HIPPOCAMPAL NEURONS. Y. L. Smith-Swintosky and M. P. Mattson. Sanders-Brown Research Center on Aging and Department of Anatomy and Neurobiology, University of Kentucky, Lexington, KY 40536.

There is considerable evidence that nerve growth factor (NGF) promotes the survival and maintenance of specific populations of sympathetic, sensory, and CNS neurons. Recent data suggest that NGF can protect/rescue CNS neurons from insults such as hypoglycemia and ischemia, including neuronal populations (e.g., hippocampus) previously believed to be unresponsive to NGF (Neuron 7:1031, 1991). Previous studies identified both a low affinity 75 kDa NGF receptor and a high affinity receptor which is identical to the txa proto-oncogene product. Both types of NGF receptors have been proposed to be important for the biological actions of NGF. Immunocytochemical and Western blot studies using polyclonal antibodies to the 75 kDa NGF receptor (REX) and txa (generous gifts from G. Westkamp, F. Lefebre, and L. Reichard) demonstrated the presence of these proteins in neurons in embryonic rat hippocampal cell cultures. In order to determine which NGF receptors might mediate actions of NGF in hippocampal neurons, we employed antiserum oligonucleotides (AO) directed against rat 75 kDa NGF receptor or txa (sequence provided by D. Clay). Levels of the 75 kDa receptor and txa were reduced in neurons exposed to the respective AO's. Administration of AO's for either NGF receptor resulted in neuronal death. However, neuronal death was more rapid in cultures exposed to txa AO (< 24 hr) as compared to 75 kDa NGF receptor AO (2-3 days). The NGF receptor AO's also eliminated the neurite outgrowth-promoting effect of NGF in glial-deprived cultures. Taken together, our data suggest that hippocampal neurons in culture possess both the low affinity NGF receptor and txa. Both NGF receptors may play a trophic role and protect hippocampal neurons against environmental insults. (NHI and Alzheimer's Association support).

401.21 INFECTION OF DISSOCIATED COCCHEAL GANGLION CELL CULTURES WITH HSV-1 and txa CHARGES THE SURVIVAL RESPONSE OF AUDITORY NEURONS TO EXTERNAL NGF. J. L. Van Der Wateren, F. P. Lefebre, L. J. Liu, K. Y. G. Moonen, J. A. Kessler, and H. Nakagawa. Dept. of Otolaryngology, Neurosciences, Medicine & Neurology, Albert Einstein College of Medicine, Bronx, NY 10461, Dept. of Physiology, University of Liege, B 4000 Liege, Belgium.

Auditory neurons in cell cultures of dissociated cochlear ganglia of the mouse after embryonic day 13 are not responsive to the survival promoting effects of nerve growth factor (Lefebre et al, 1991; Acta Otolaryng. 111:301-311). Transfection of PC12 cell mutants deficient in high affinity NGF binding site with full length rat txa cDNA restored the responsiveness of these PC12 cell line to NGF (Lueb et al, Cell 66:961-966, 1991).

A defective herpes simplex virus-1 vector (HSV-1) containing a gpt (HSV-txa) was used to infect cultures of dissociated cochlear ganglia that contained auditory neurons that were unresponsive to NGF. The expression of gpt 14OPT was driven by the HSV IE 6/5 promoter so that all infected cells of the culture would express the gpt 14OPT construct. When infected cultures were compared to uninfected ones, a significant enhancement of the survival of auditory neurons in the gptHSV-1 infected cochlear ganglion cell cultures treated with exogenous human recombinant NGF (1, 100 ng/ml) was evident in contrast to the uninfected control cultures treated with hGF.

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402.1 EXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-4 AND -5 mRNAs IN ADULT RAT BRAIN. Kaye L. Steinhurt*, Michelle Gudjons, Ellen M. Zemlan, and Ronald腾 Chen. Salk Institute for Biological Studies neurobiology and Dept. of Medicine, University of North Carolina, Chapel Hill, NC 27599.

Insulin-like growth factors (IGFs) are believed to serve as neurotrophic factors in developing and adult rat brain. The IGPs are associated with a family of at least six IGF binding proteins (IGFBPs) which prolong the half-life of plasma IGFs and modulate IGF actions. Although high levels of IGF binding proteins are present in adult rat brain, their cellular sites of synthesis are still poorly characterized. To localize sites of IGFBP-4 and -5 mRNA expression, we performed in situ hybridization histochemistry using 35S-labeled antisense riboprobe to one of the IGFBP-4 and -5 mRNA provided by Drs. Nicholas Ling and A. D. Dufour, respectively. We examined fresh-frozen, post fixed sections of adult rat brain. Specificity of hybridization was confirmed by negative results in sections hybridized with sense riboprobe pre-treated with RNAse A prior to hybridization. The two IGFBP mRNAs were abundantly expressed within discrete regions of brain. Generally, the same expression patterns of the two mRNAs were non-overlapping. Notably, IGFBP-4 mRNA was highly expressed within hippocampal and cortical areas, whereas IGFBP-5 mRNA was not detected above background in these areas. Within hippocampus, immunoreactivity of the IGFBP-4 probe was detected in pyramidal neurons of a medial segment of the CA1 subfield and throughout the CA2 subfield of Ammon's horn. Strong immunoreactivity was also detected within the indusium griseum and subiculum. In cortex, IGFBP-4 mRNA was widely expressed in most cortical areas and layers. In contrast, IGFBP-5, but not IGFBP-4 mRNA was detected within fasciculus dentatus. The distinct expression patterns of IGFBP-4 and -5 mRNAs within brain suggest that these IGFBPs may mediate compartmentalization of the IGFs or paracrine/autocrine actions of the IGFs within the brain regions. Furthermore, expression of IGFBP-4 mRNA within hippocampal pyramidal neurons suggests a role for IGFBP-4 in modulating neuronal effects of the IGFs.

402.2 ASSOCIATION OF INSULIN-LIKE GROWTH FACTOR 1 (IGF1) ON BASAL FOREBRAIN CHOLINEURGIC NEURONS. A. Beauregard*, C. Desgrange, and M.-P. Faure. Neuroanatomy Lab, Montreal Neurological Institute, McGill University, Mil, Que, Canada H3A 2B4.

IGF1 was previously shown to stimulate the release of acetylcholine in adult rat brain slices. It is unknown whether IGF1 exerts its effects directly on central cholinergic neurons. To test the hypothesis that IGF1 receptors may be associated with basal forebrain cholinergic cells, we examined by double immunofluorescence, the labeling of IGF1 and ChAT in rat medial nuclei. Primary antibodies were reacted with secondary antibodies conjugated with FITC and Texas Red, respectively. In the medial septal nucleus, diagonal band of Broca and substantia innominata a large proportion of ChAT positive neurons exhibited IGF1 immunoreactivity. Reconstruction of serially sectioned images using confocal laser microscopy (CLSM) substantiated that IGF1 and ChAT immunoreactivity were present in a number of basal forebrain cells. Confocal microscopic analysis of this pattern of IGF1 was mostly membranous and took the form of highly fluorescent punctate dots. Hybrid cells derived from septal cholinergic neurons, produced by the fusion of basal forebrain cells with murine neuroblastoma cells, were used to characterize the biological effect of IGF1. The hybrid cells were treated for different times and concentrations with nerve growth factor, basic fibroblast growth factor, platelet-derived growth factor and IGF1. Stimulation with IGF1 (10 ng/ml) for 48 hours produced a change in cell shape and significant neurite outgrowth (15-25m) whereas none of the other factors produced any effect under these conditions. Moreover, fluorescence ChAT immunoreactivity was evaluated by CLSM and expressed a 10 fold increase following IGF1 stimulation. These results suggest that IGF1 may play an important role in the growth and function of basal forebrain cholinergic neurons and, consequently, that the impairment of its interaction with IGF1 may be involved in the degeneration of cholinergic systems such as occurs in Alzheimer's disease.
A RAPID INCREASE IN ACTION POTENTIAL FIRING RATE IN RESPONSE TO GROWTH FACTORS. R.H. Salwaye and L.A.C. Blair. Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

The role of growth factors in the adult mammalian central nervous system is unknown. We have examined the rapid modulation of action potential frequency and ionic currents by growth factors known to be present in the central nervous system. Patch clamp recording techniques were used to indentify spontaneous electrical activity and ion channel events in GHSa-pituitary cells. It was found that culturing in serum-depleted conditions blocked the firing of spontaneous action potentials. Brief exposure to serum or to a pool of 14 growth factors normally contained in serum fully reconstituted electrical activity. Subsequent experiments demonstrated that addition of insulin, IGF-I and IGF-II are sufficient to restore normal action potential behavior. Specifically, using on-cell single channel recordings or whole-cell recordings in the current clamp configuration, the reconstitution of electrical activity was rapid (requiring 30 seconds to 5 minutes) and stable. Furthermore, preliminary whole-cell voltage clamp analysis suggests that both outward potassium currents and inward calcium currents are altered by the presence of growth factors. One of these growth factor-modulated currents is tetraethylammonium-sensitive but insensitive to 4-aminopyridine, indicating that Kv1 may be one of the potassium channels regulated by growth factors. Together, these experiments suggest that the insulin family of growth factors may be capable of inducing rapid changes in channel calcium and one or more potassium channels.


We have studied the presence of insulin like growth factor I (IGF) and its receptors in 22 day and 18 day gestation neuron cell cultures (NCC) from fetal rabbit brains. The 22 day NCC were incubated in IGF free/free serum free medium (ISFM) and the 18 day NCC in serum medium. The 18 day NCC died in an ISFM. The peroxidase anti-peroxidase method using rabbit anti-IGF antibody (R configuration) (1/100)(E.Lilly) showed IGF present. Antibody absorbed with IGF(E.Lilly) and rabbit serum lacked immunoreactivity. In situ hybridization using a 32P -biotinylated oligonucleotide revealed IGF mRNA. Electron microscopy using RIGF (1/10000) and anti-neuronfilament (1/10000) showed IGF in the neurons' endoplasmic reticulum, Golgi, cytoplasm and prolongations. IGF had enhanced neuron growth in ISFM. 10 day NCC from 18 day culture were transplanted into 15 day ISFM. We conclude: A) fetal NCC produce IGF, B) IGF promotes cell survival, and C) exogenous IGF may be needed in early brain development.


Recent studies have indicated that insulin like growth factor I (IGF-I) and IGF-I and IGF-I receptor mRNA are abundant in both the developing and in the mature rat brain olfactory bulbs (Ay-er Lieve et al., 1991; Wether et al., 1990, Bandy et al., 1992). By using the method of intracoriaral transplantation we were able to study effects of truncated IGF-I (IGF-I), antibodies against IGF-I and IGF-I binding protein on developing olfactory bulbs. 116 olfactory bulbs were grafted to the anterior chamber of adult host rats. Grafts were treated with either 20 µg/ml IGF-I, 1 µg/ml anti-IGF-I, 1 µg/ml IGF-I binding protein or vehicle solution alone prior to grafting. Similar results were obtained by intracoriaral injections S, 10, and 15 days postgrafting. Growth of grafts was monitored by direct observation and measurement through the cornea of the living animals. Grafts treated with IGF-I antibody showed growth similar to grafts receiving only saline treatment. When similar experiments were carried out on E16 and E17 partical cortex grafts, no enhancement in graft size was seen after IGF-I antibody treatment. However IGF-I has in previous studies (Gacubini et al. 1990) been shown to enhance growth of intracoriarally transplanted fetal partical cortex grafts. These results indicate that IGF-I is important in development in a regionally specific manner. Immunohistochemical studies of the olfactory bulb grafts are currently under investigation.

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) INCREASES RATE OF FUNCTIONAL RECOVERY FROM SCIATIC NERVE CRUSH IN MICE. P. C. Contreras*, C. Steffler and J. L. Vaught. Pharmacology, Cephalon, Inc. West Chester, PA 19380.

IGF-I has been shown to stimulate several growth-associated processes and to support the survival of α-motor neurons in vitro. IGF-I has also been shown to enhance neuronal sprouting after sciatic crush in rats. The purpose of this study was to assess whether IGF-I also increases rate of functional recovery after sciatic nerve crush in mice. After Ski-Webster mice (25-35 g) were anesthetized, both sciatic nerves were exposed and crushed for 10 sec with a hemostat, covered with plastic tubing. Mice were injected with vehicle (1% BSA) or IGF-I (1mg/kg) s.c. after recovery from the anesthetic and for the next 15 days. Functional recovery from the sciatic crush was measured by 1) determining the number of times/5 trials the mice were able to grasp an inverted screen with both hindpaws; and 2) assessing changes in gait. Mice treated with IGF-I were able to grasp the inverted screen and return to normal running pattern but not the vehicle treated mice. There were also parallel improvements in several parameters used to measure gait, such as toe spread, in IGF-1-treated mice compared to vehicle-treated mice. These results indicate that IGF-I, which enhances survival of α-motor neurons, also enhances functional recovery. These data support the utility of rIGF-I for the treatment of ALS.
402.1 BASIC FGF AND FGF RECEPTOR-4 OCCUR IN CHICKEN. P. C. Evers and M. Bothwell*. Dept of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

The fibroblast growth factor (FGF) family presently consists of 7 members: aFGF, bFGF, Int-2, k-FGF, FGF-5, FGF-6, and KGF. These ligands bind, with specificity which is not fully defined, to mitogenic receptors of 4 genes: FGF receptor-1 (FGFR1), FGFR2, FGFR3, and FGFR4. Our previous results show FGFR1 expressed in the developing chick neurepithelium, where it is likely to have a role in proliferation and differentiation. To further explore the role of FGFs in neural development, we plan to describe the expression of the FGFs and other FGFRs in neural tube. Hence, we have sought to clone segments of each FGF and FGFR gene for use as in situ hybridization probes. Portions of six genes: aFGF, bFGF, FGFR1, FGFR2, FGFR3, and FGFR4 from chicken using PCR and degenerate nucleonucleotides. The sequences for two of these genes, bFGF and FGFR4, have not been reported previously. Sequence identity between the putative chicken bFGF and the mouse bFGF is 83% at the nucleotide level and 94% at the amino acid level. The nucleotide sequence identity between the putative chicken FGFR4 and the three known chicken FGFR genes is 72-79%, whereas the nucleotide sequence identity between the putative chicken FGFR4 and the human FGFR4 is 79%. Sequence comparisons suggest that the two cDNAs are portions of the bFGF and FGFR4 genes in chicken. The bFGF and FGFR4 cDNAs along with the cDNAs for aFGF, FGFR1, FGFR2, and FGFR3, will be useful in determining the roles of FGF in neural development.


Fibroblast growth factors (FGFs) are potent growth factors with roles ranging from development to adult plasticity in the brain. FGF-5 is a new member of the FGF family with a potential role in brain function and pathology. In order to evaluate a possible role of FGF-5 in the brain, we have examined the locus of synthesis of FGF-5 in the rat brain using in situ hybridization of S35-labeled RNA probe complementary to FGF-5 mRNA. FGF-5 mRNA was expressed in neurons in selected regions of the rat brain. FGF-5 mRNA in situ hybridization labelling was particularly intense in the olfactory bulb within periglomerular elements and granular cell layer. The primary olfactory cortex also showed strong FGF-5 mRNA labelling and mostly within cell layer II, throughout its rostro-caudal extent. In the hippocampal formation, the greatest intensity of FGF-5 mRNA labelling was shown in hippocampal pyramidal cells within subfields CA3 and CA2, and granular cells within the dentate gyrus. The cerebral cortex (neocortex) showed a modest labelling throughout its rostro-caudal extent, mostly within external layers. The entorhinal cortex showed a slightly higher labelling intensity as compared to the neocortex. The cerebellum, thalamus and striatum displayed light labelling. Gial-like cells scattered throughout the brain appeared to express low levels of FGF-5 mRNA. In general FGF-5 mRNA was mostly shown by limbic structures, suggesting the possibility that FGF-5 may play a role in limbic system function or pathology.

403.3 BASIC FIBROBLAST GROWTH FACTOR (bFGF) GENE EXPRESSION IN RAT BRAIN: AGE AND REGIONAL COMPARISON BY QUANTITATIVE RT-PCR. A. El-Hajjim, L. Apenten*, and R.P.C. Shih. Departments of Physiology and Anatomy, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0W3.

Investigations in vitro have shown the importance of bFGF as a mitogen for astrocytes and as a neurotrophic factor for many neuronal types. This study was designed to compare bFGF gene expression in selected regions of the brain of rats at different postnatal ages. The reverse transcription-polymerase chain reaction (RT-PCR) was used to measure the levels of bFGF mRNA. These levels were quantitated relative to the levels of mRNA for glyceraldehyde-3-phosphate dehydrogenase, the latter being constant. We first compared the levels of bFGF mRNA in the cerebrum from male rats of ages 1, 3, 7, 14, 21 and 28 days and one year. Our results showed that by the end of the first month, the cerebral bFGF mRNA was about 10-fold that of the 1 day old rat, with the greatest increase occurring between the first and the second postnatal weeks. One year old cerebrum showed high levels of bFGF mRNA similar to that of 21 day old. Analysis of different regions of the 28-day old brains revealed that the lowest levels of bFGF mRNA occur in the cerebellum, and that the highest levels occur in hippocampus (7 times the levels in cerebellum, 7X), followed closely by cingulate cortex, occipital cortex, and inferior colliculus (4-5X). The highbathalumus and combined pons-medulla showed intermediate levels (2X). Preliminary studies of these regions from 1 day old rats showed there was a different pattern of regional expression at this age. Our results are consistent with the hypothesis of a role of bFGF in brain development.


Recently, it was shown that basic fibroblast growth factor (bFGF) is present in distinct neuronal subpopulations of the brainstem (Grothe et al J. Comp. Neurol. 323:119-121, 1991). Immunohistochemical analysis revealed a partial overlap of bFGF and neuropeptides (5HT, SP, CGRP) or tyrosine hydroxylase in some brainstem nuclei but no strict co-localization of bFGF with one of the neuropeptides or tyrosine hydroxylase.

To identify bFGF-responsive cells in the brain, immunocytochemistry and binding studies were performed. FGF-receptor could be localized in the cortex and, like bFGF, in neuronal subpopulations of several motor and sensory brainstem nuclei.

After colchicine treatment bFGF-IR disappeared in brainstem nuclei expressing the FGF-receptor and appeared or increased in nuclei lacking the FGF-receptor. Whether this change in the bFGF-IR is exclusively due to transported bFGF has to be clarified by in situ hybridization.

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We have shown that bFGF-immunoreactivity (IR) is present in a neuronal subpopulation of postnatal and adult crest- and placode-derived sensory ganglia (Weise et al. Cell Tissue Res., 267: 125-130, 1992). In dorsal root ganglia (DRG) bFGF was strictly co-localized with the somato-statinIR subpopulation. Northern blots of total RNA from DRG and cortex revealed 3.7 and 3.9 kb mRNAs. In situ hybridization showed that the bFGF mRNA is present in nearly all DRG neurons. To identify neurons with putative bFGF responsiveness, FGF-receptor immunocytochemistry and binding studies were performed. The FGF-receptor IR was present in all bFGF-immunoreactive neurons suggesting that the protein mediates its effects in an autocrine and/or paracrine manner. The possibility that the distribution of the FGF-IR is different in the distribution of bFGF-IR and bFGF mRNA may include: 1) different translational regulation in distinct subpopulations; 2) different stabilities of mRNA and proteins.

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Fibroblast growth factors (FGFs) are a family of related peptides which have been characterized by their ability to influence cellular differentiation and their mitogenic/angiogenic properties. We have observed the acidic and basic FGF mRNA and protein in sensory and motor neurons of the rat including the substantia nigra (SN). In contrast to the widespread distribution of basic FGF and acidic FGF expression is quite restricted. We examined the expression of aFGF and bFGF in the rat SN and dorsal root ganglia (DRG) from E16-18 to P90.

In DRGs, aFGF mRNA and protein were present at E18 and were observed through adulthood. bFGF mRNA was present in sensory and motor neurons of the rat. aFGF mRNA was first detectable in the SN at PN20 and was expressed through adulthood. The developmental expression of acidic and basic FGF in the SN may indicate different functional roles in this brain region.
403.7 BASIC FIBROBLAST GROWTH FACTOR (bFGF) PROMOTES THE SURVIVAL AND PROLIFERATION OF MESCENEPHALIC NEURONAL PRECURSORS IN VITRO. M.M. Bouvier1 and C. Mytilineou. Dept. Neurology, Mt. Sinai Sch. of Medicine, New York, N.Y., 10029.

We recently demonstrated that treatment with epidermal growth factor (EGF) results in the survival and proliferation of neuronal and glial stem cells from embryonic day 16 (E16) rat mesencephalon (Mytilineou et al., 1992). To determine whether dopaminergic neuronal precursors could also be induced to proliferate in vitro, we used ventral mesencephalic cultures from E12 rat embryos, a developmental stage that coincides with the beginning of the ventral mesencephalon. Mesencephalic cultures were treated with EGF (10ng/ml), bFGF (10ng/ml) or a combination of EGF and bFGF at the day of plating. Control and tropic factor-treated cultures were observed with phase contrast microscopy and analyzed at 7 days in vitro (DIV) by immunocytochemistry with antibodies to neuron specific enolase (NSE), tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) to identify differentiated neurons, dopaminergic neurons and astrocytes, respectively. Some cell division, resulting in colony formation, occurred in the first 24-48 hrs after plating at all tropic factor-treated cultures, as well as in untreated controls. However, treatment with bFGF resulted in greater cell number and the formation of larger colonies. EGF had no apparent effect in these cultures. Cell loss was prominent in control and EGF-treated cultures after 7DIV, but it was less apparent in cultures treated with bFGF. At 7DIV the majority of surviving cells were positive for NSE while <1% stained with GFAP. TH immunocytochemistry revealed clusters of dopaminergic neurons in control and EGF-treated cultures, but their numbers were higher after treatment with bFGF. (Supported by NIH grant NS-23017 and the United Parkinson Foundation).

403.9 EFFECTS OF BASIC AND ACIDIC FGF ON GROWTH AND DOPAMINE NERVE FIBER PRODUCTION OF INTRAUTERINALLY TRANSPLANTED FETAL MESCENEPHALIC GRABS. S. Almqvist1, Z. Almqvist2,1, Y. Car1, R. Pettersson1, J. Olson1, Dept. of Histology & Neurobiology, Karolinska Institutet, Stockholm, Sweden, 2Fudan University, Shanghai, China.

Basic FGF has been shown to increase dopamine neuron survival in mesencephalic cultures (Ferrari et al. 1988) as well as inducing regrowth of damaged DA neurons in vivo (Otto, Unencer, 1990). We have followed the growth and survival of developing E14-E16 mesencephalic cultures under chronic intermittent treatments with either aFGF or bFGF in the anterior eye chamber of adult rat hosts. Pieces to be grafted were cultivated in either 25 μg/ml aFGF, 25 μg/ml bFGF or vehicle solution alone prior to grafting and 5 μl of similar solutions were injected intravitreally on days 5, 10 and 15. Host animals were sympathetically denervated 2 weeks prior to grafting enabling evaluation of catecholaminergic fiber outgrowth upon the host iris in whole-mount preparations by use of the Falck-Hillarp technique. Both aFGF and bFGF significantly increased the volume of transplanted mesencephalic grafts when compared to grafts treated with the vehicle alone. bFGF was a more potent growth stimulator than aFGF. Both bFGF and aFGF increased the area of the host iris innervated by graft-derived catecholaminergic containing fibers. Immunohistochemical evaluations of grafts are currently under study as well as studies of treatment of mesencephalic grafts using the described model with other growth factors.

403.11 THE COMBINED ACTION OF BASIC FGF AND SUBSTRA PROMOTES DIFFERENT CHROMAFFIN CELL FATE. C.H.Chu,1,2 C.O. Crabtree,1 A.M.Tolikovsky, Dept. of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, U.K.

Basic fibroblast growth factor (bFGF),like NGF, induces cell division and neurite outgrowth in adrenal chromaffin cells. In vitro, we found that 3H-thymidine incorporation of the adrenal chromaffin cells was increased by bFGF, unlike NGF, which promotes different neurite outgrowth responses on neural retinal chromaffin cells with different substrata. To determine the extent of chromaffin cell transformation into neurons, we counted the number of cells with tyrosine hydroxylase positive staining and neurites and cell division was assessed by 3H-thymidine incorporation of the chromaffin cells. The percent of cells expressing 3H-thymidine incorporation of the chromaffin cells was significantly reduced on laminin compared with collagen Type I. In contrast, cultures grown on collagen Type I, bFGF elicited little neurite outgrowth, and promoted the proliferation and survival of chromaffin cells. In addition, dexamethasone induced neurite outgrowth in response to bFGF on laminin and increased the proportion of surviving and tyrosine hydroxylase positive cells. These results show that bFGF and laminin act synergistically to promote sympathetic neuronal transformation, whilst on collagen Type I or in the absence of bFGF, bFGF acts as a mitogen and survival factor of chromaffin cells.


Basic FGF is expressed in catecholaminergic cells of substantia nigra and in sympathetic-adrenal system. We have recently shown that in adrenomedullary (AM) cells exogenous bFGF modulates expression of tyrosine hydroxylase and dopamine beta hydroxylase genes, suggesting that it may serve as an autocrine or paracrine regulator in catecholamine and norepinephrine biosynthesis. The present work was undertaken to determine subcellular localization of bFGF in AM cells and to examine whether bFGF expression is regulated by stimulus that affect synthesis of AM hormones. In cultured bovine AM cells stimulation with bFGF against recombinant bFGF, granular cytosolic and nuclear bFGF. Immunoreactivity (bFGF-IR) was observed. In majority of cells bFGF staining was more intense in the nucleus than in the cytoplasm. Incubation with exogenous 18 kDa bFGF (5x10^{-14}M) led to an increase in nuclear bFGF-IR within 10 min, which reached a maximum between 1-3 hrs. Incubation with forskolin produced a dramatic increase of nuclear bFGF-IR which peaked at a maximum after 12 hours. Lower pronounced increases in nuclear staining, were observed in cells treated with angiotensin II or with the depeolarizing agent, veratridine. Western blot analysis of total AM cells with antibodies against bFGF demonstrated that bFGF-IR was expressed in AM cells by hormonal (angiotensin) and neural (depolarization) stimuli, and by 2nd messenger (cAMP), supports the hypothesis that bFGF plays an active role in the plasticity of the adrenergic system. It also suggests that long-term genic effects of bFGF may be mediated directly in the nucleus.
404.3 MITOGENIC EFFECTS OF ACIDIC FIBROBLAST GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR BETA ON BOVINE AND PORCINE ENDOTHELIUM IN VITRO. T.C. Ryken and V.C. Travenlis*. Department of Surgery, Division of Neurosurgery, University of Iowa College of Medicine, Iowa City, Iowa, USA.242.

The mitogenic effects of acidic fibroblast growth factor (aFGF) and transforming growth factor beta (TGF-beta) were assayed alone and in combination on cultured bovine and porcine endothelium. Triplicate growth-arrested cultures of each cell type were grown in the presence of aFGF (300 ng/ml) and/or TGF-beta (5 ng/ml) for 96 hours, renewing the media each 24 hours. The mitogenic effects were assayed by cell counting at the conclusion of the 96-hour period. Results are reported as a stimulation index of the initial cell number.

Both bovine and porcine endothelial cell cultures incubated with aFGF underwent a marked increase in mitogenic activity, increasing in stimulation index to 1.60 (± 0.05) for control (1.0 ± 0.0) and 3.81 (± 0.06), respectively. Endothelium incubated in the presence of TGF-beta underwent a decrease in mitotic activity with a stimulation index of 1.16 (± 0.10) and 1.17 (± 0.05) in the bovine and porcine cultures. Cells incubated in a combination of aFGF and TGF-beta demonstrated an attenuation of the mitogenic effects observed in the presence of aFGF alone, decreasing the stimulation index to 3.25 (± 0.11) in bovine endothelium and 1.80 (± 0.30) in porcine endothelium.

These results suggest that the mitogenic effect of aFGF in cultured endothelium can be antagonized by TGF-beta. The interaction of growth factors on endothelium is of interest to elucidate the mechanisms involved in angiogenesis and transformation.

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Basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) are multifunctional proteins for neurons and astrocytes in culture. The mechanisms regulating the expression of bFGF and NGF in neurons and astrocytes of developing or injured rat brain are not well understood. Previous work demonstrated that hydrogen peroxide induces bFGF, NGF and c-fos in astrocytes. It has been shown that excitatory amino acids (EAA) induce calcium influx in hippocampal neurons.

In this study we demonstrate that EAA treatment increases c-fos and growth factor mRNA in a time dependent manner. When neurons and astrocytes were incubated in the presence of DNXQ and APV the expression of the mRNA was reduced. These findings suggest a possible interaction between glu-receptor activation and growth factor expression as an important aspect of neuronal-glial communication.


Effects of human recombinant acidic fibroblast growth factor (hhaFGF) on brain neurons of various regions in primary culture and hippocampal-lag long-term potentiation (LTP) in rat brains were examined. Dissociated cells from 8 regions of embryonic day 16 rats were cultured in medium containing 10% serum for 1 day and chemically-defined serum-free medium with hhaFGF for 3 days. 10-100 ng/ml hhaFGF enhanced neuronal survival in the cortex, hippocampus and substantia nigra, while 10 ng/ml CS23 (modified human basic FGF) was effective in all regions tested. Next, effects of hhaFGF and CS23 to the increases of the spike amplitude induced by tetanic stimulation were measured in the dentate gyrus of 24 hr fasted and non-fasted rats. Ten ul of drug was i.c.v. injected before the application of tetanic stimulation. hhaFGF (400 ng/ml) didn't influence the LTP induced by the tetanus of 100 pulses at 100 Hz in both fasted and non-fasted rats, but significantly facilitated the generation of LTP by tetanus of 20 pulses at 60 Hz only in fasted rats. However, 400 ng CS23 induced LTP when the tetanus of 20 pulses at 60 Hz was applied in both fasted and non-fasted rats. Protein kinase C activities of hippocampal cytosol fraction was decreased with the i.c.v. injection of 400 ng hhaFGF in 24 hr fasted rats but not in non-fasted rats. These results suggest that hhaFGF might be one of factors of feeding and memory and there might be different regulatory mechanisms on FGF receptors from those of basic FGF.

404.2 STAGE AND CONCENTRATION SPECIFIC EFFECTS OF RETINOIC ACID ON THE DIFFERENTIATION OF XENOPUS MINDRAIN AND EAR. T. Neary* and B. Fritzsch. Anatomy Div., Creighton University, Omaha, NE 68178.

Retinoid acid (RA) causes developmental defects of certain brain areas (Papapolu et al., Development 113 (1991) 1145) that could result either from abnormal inductive interactions between mesoderm and neuroectoderm or from direct effects of RA on the developing CNS. To discriminate between these possibilities we exposed Xenopus laevis embryos (stages 15-21; from completion of gastrulation to completion of neurulation) to 30 min. RA pulses (5 x 10^{-7} - 5 x 10^{-5} M RA). Reticulospinal projections, known to be altered by RA (Manns and Fritzsch, Neurosci. Lett. 136 (1992) 1) and connections of the inner ear were examined in later stages with fluorescent dextran amines. Effects on the reticular formation, (supernumerary Mauthner-like cells) were found with increasing concentrations of RA as late as st 21. In the ear, the separation of the utricular and saccular macula and the formation of the horizontal canal is suppressed with exposure to increasing concentrations of RA as late as st 21. Reductions in the numbers of sensory ganglia and efferent cells were concomitant with this effect. These data suggest that RA has a direct effect on the CNS and inner ear. Supported by Health Future Foundation.
404.3
A MONOCLONAL ANTIBODY THAT RECOGNIZES A NOVEL POSITIONALLY-REGULATED EPITOPE IN THE RAT CNS
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In an attempt to generate mAbs against downstream products of homologues expressed on neuronal surfaces, we employed NTD21
embryonic carcinoma cells. Retinoic acid induces homoblasts gene
expression and a neuronal phenotype in these cells. After the mouse's immune response to the NTD21 cell membrane is suppressed with
cyclophosphamide, membranes from the same cells treated with retinoic acid were injected. Hybridomas supernatants from this fusion were
screened on the NTD21 cell line and selected with specific hybridoma cell lines. mAb 5T6 (olfactory-tenectelephalic) staining is detected from E9 to E15 in the prosencephalon and part of the diencephalon.
The only other sites of staining are the nasal pits and the optic placodes. The rest of the nervous system and all non-neural tissues are
negative. At ED, staining also appears in the glomerular layer of the olfactory bulb, the olfactory epithelium, and the lateral nasal gland. From E16 through PI, limited staining is also detected in some regions of the
cortex and thalamus. In the adult, the entire brain is positive.

Immunoblotting identifies high molecular weight, proteoglycan-like antigens with a tissue distribution consistent with the
immunohistochemical localization at E14.5.

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404.4
THE MURINE HOX-1.2 GENE CONTAINS A CNS REGION SPECIFIC
REGULATORY DOMAIN.

The patterns of Hox gene expression during mammalian central nervous system development are remarkable for their spatial restriction, within a cluster of Hox genes, each gene has a more rostral extent than does its immediate 5' neighbor. We have begun a study of the homeostructures I gene to investigate mecha-
nisms of this intriguing spatial control of gene expression.

We have examined two lines of transgenic mice bearing 3.7 kbp of the Hox-1.2 gene upstream of the transgenic start site found on the B. coli 8.8 kb transgenic reporter gene. At embryonic day 12.5 there are high levels of reporter protein staining in the doral medulla, conforming to the endodermal. Staining appears to be localized to a small group of neuroblasts, which we have provisionally identified as the nucous nucleus solitus (NNS) which may be supported by labeling of this region by the carboxyanine dyel applied to the glossopharyngeal/vagal ganglia.
Interestingly, the vagus, trigeminal and facial nerves and ganglia are also stained. These latter nerves also have projections to the NTS, suggesting that Hox genes may participate in the specification of functionally integrated neural pathways.

The reporter gene is also expressed in the ventral horns of the caudal spinal cord and in a few lateral cells of the thoracic dorsal root ganglia. In one transgenic strain, at embryonic days 13 to 15, virtually the entire peripheral nervous system is stained, including the sympathetic chain and ganglia. In this same strain, reporter expression persists into adulthood, with prominent staining in the granular layer of the cerebellum, pontine nuclei and the dorsal horn but not the ventral horns of the spinal cord. Our results demonstrate that one Hox gene has temporally regulated expression in several distinct and nonoverlapping regions of the CNS. Therefore, expression of these genes is not confined to a single timepoint or single region of the neuraxis, although they may participate in positional or functional specification within these areas.

Supported by NIH K08 NS01464-01 and March of Dimes #5-FY91-0702 to JG.

404.5
EVIDENCE FOR LINKAGE OF TES-1 AND DLX-1, TWO HOMEOBOX GENES EXPRESSED IN THE DEVELOPING MAMMALIAN FOREBRAIN.

Tes-1 and Dlx-1 are members of the Distal-less family of
homeobox genes and are likely candidates for regulating positional identity or cell differentiation in the developing forebrain. Tes-1 and Dlx-1 are both transiently expressed in the
cells of the embryonic ventral forebrain that give rise to the
temporal, entorhinal, thamic nuclei, and olfactory bulb. The present studies used pulsed-field gel electrophoresis (PFGE) to determine if Tes-1 and Dlx-1 are physically linked to each other, in the case for the Hox genes. Mouse thymus (A9) genomic DNA aliquots were digested with nine rare-
cutting restriction enzymes, subjected to PFGE, transferred to nylon filters, and sequentially hybridized to 32P-labeled Tes-1 and Dlx-1 cDNA probes. The probes co-hybridized to fragments generated by the four restriction enzymes: Nru I, Not I, Sal I, and BsuI. The physical linkage was further demonstrated by co-hybridization of the probes to DNA fragments generated by digestion with combinations of restriction enzymes.

The smallest DNA fragment (generated by digestion with Sfi I and Not I) that was recognized by both probes was approximately 45 kb. These results indicate that Tes-1 and Dlx-1 are physically linked within approximately 45
kb on the genome. These two genes may exist as part of a larger gene complex that acts in a coordinated fashion to regulate forebrain development.

404.6
GENETIC CONTROL OF SEGMENT-LIKE PATTERNS OF GENE
EXPRESSION IN THE MOUSE CEREBELLM.
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We have previously shown that the gene for a Purkinje cell-specific
marker, L7, can be used to drive expression of β-galactosidase in
cerebellar Purkinje cells of transgenic mice. Although all Purkinje cells
in the adult express both the transgene and endogenous gene, early
developmental expression of both reveals a series of positive and
negative bands. The number of bands increases in the medio-lateral
direction during development until all Purkinje cells are positive.

Truncation of the promoter region of the transgene disrupts this normal developmental pattern. Animals carrying the truncated constructs show precocious lateral expression in early development. There is also a delay in the normal "filling in" of the bands. This suggests a series of positive and negative responders which are important for control of the banding pattern. In addition, these truncations have identified a minimal promotor sufficient for driving Purkinje cell-specific expression. This small promotor region contains a cluster of consensus binding sites for known families of developmental control
geneces and these sites can be footprinted by purified proteins from these families or by crude cerebellar nuclear extracts. By specifically
mutating these elements we are assayng their affect on cell-specific transgene expression. We are using a FCR strategy to identify the
cerebellar transcription factors themselves.

404.7
A MOLECULAR APPROACH TO CEREBELLAR
COMPARTMENTATION: THE CLONING OF ZEBRIN II
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The Zebrians are the first example that represent striking examples of the parasagittal organization of the cerebellar cortex. Zebrian staining by immunohistochemistry appears as sharp anterior to posterior border and like ventral and lateral (or less well registered bands) in the hemispheres. The bands have been likened to "compartmental" and probably reflect a basic organizing principle of the cerebellum. To study the molecular genetic basis of the Zebrian periodic pattern of expression, we have cloned Zebrian II, a 32 kd intracellular antigen, from a cDNA expression library of postnatal day 20 C57BL/6J mouse cerebellum. Several independent recombinants express a single class of cDNA recognizing by the Zebrian II monoclonal antibody. Partial sequence from these clones reveals a near identity to that previously published for rat and human aldolase C, one of the three
known aldolase isozymes. Aldolase is a glycolytic enzyme that hydrolyzes fructose 1,6-diphosphate, and previous reports show that the expression of aldolase C is heterogeneously restricted to Purkinje cells of the cerebellum; no higher-order organization of its expression has been previously recognized. To signal the whether the HAND pattern of Zebrian/aldolase antigen in cerebellum was due to differential transcription, in situ hybridization of horizontal sections of mouse brain was performed. Immunostaining appears in the cerebellar Purkinje cell layer, with regional heterogeneity. The registration of this heterogenous pattern with that of Zebrian II bands will be assessed.

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404.8
FURTHER CHARACTERIZATION OF MEANDER TAIL, A GENETIC
MUTATION AFFECTING CEREBELLAR MORPHOGENESIS. C.

We have previously described the effects of a genetic mutation on the cellular and foliar structure of the mouse cerebellum (Ross et al., PNAS 87:4189, 90). We now present further analyses of this phenotype, including a three dimensional reconstruction of the mutant cerebellum. This analysis demonstrates that a rather large volume, comprising the "anterior" cerebellum, is completely missing. Thus, the meander cerebellum is a posterior half-cerebellum. We have also analyzed the meander tailed phenotype. There is a progressive failure of organization, with altered foliation and glial cell structure apparent at PO. The cells in the anterior external granule layer do not proliferate and the Purkinje cells never organize into a monolayer. By P7 the mutant phenotype is fully established. These results demonstrate the unique specification of the developing cerebellum.
404.10 NEURON SPECIFIC ENOLASE PATCHES IN THE DEVELOPING RAT STRIATUM. W.E. Silverman* and Y. Solomon. Unit of Morphology, Coro Center for Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel.

One of the initial events signaling the development of patch and matrix domains in the embryonic striatum is the appearance of islands or patches of dopaminergic (DA) neurons in the neostriatum. Neurons in the cells in the patches subsequently produce opiate receptors and substance-P (SP). A complementary pattern, the matrix, is produced postnatally following ingrowth of fibers from the remaining DA cells into the area between the patches, followed by synthesis of neurotransmitter, calcium binding protein and other peptides by neurons there. This sequence of neuropeptide expression preceded by the ingrowth of DA terminals suggests an active role for the mesostriatal projection. We have examined the ontogeny of immunoreactivity for neuron-specific enolase (NSE), a metabolic enzyme-marker for synaptogenesis and neuronal activity with respect to that of tyrosine hydroxylase, SP and neurotensin in the striatum and ventral mesencephalon of the developing rat at the light and ultrastructural levels. The most striking findings were that NSE appeared exclusively in striatal patches until the 2nd postnatal day, when it co-localized with SP in neuronal perikarya; by P2 NSE also appeared in neurotensin-positive cells in the matrix. The data obtained support the hypothesis that DA fibers establish connections in the striatum, and induce metabolic activity and expression of neuropeptides, initially in the patches and then in the matrix. Supported by The Israel Ministry of Science and Technology.
GLIA AND OTHER NON-NEURONAL CELLS III

405.1 GLIAL CELL RESPONSE TO SUBSTRATE-BOUND ADHESION MOLECULES. H. R. Paun* and J. Lemmon. Department of Neuroscience, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

During neurogenesis and regeneration, both soluble and noninvasive molecules may act as cues for glial cell development. We examined the influences of purified extracellular matrix molecules and cell adhesion molecules on the development and proliferation of neuroglial cells from neonatal rat optic nerve. Dissociated optic nerve glia were plated on nitrocellulose-bound fibronectin, laminin, collagen type IV, L1, n-cadherin, and NCAM. Cultures were grown in chemically-defined medium to promote formation of oligodendrocytes. Other cultures were grown in 10% serum to support type-1 astrocytes and the differentiation of progenitor cells to type-2 astrocytes. Short term adhesion assays were used to measure cell affinity for the different substrates. In these experiments, glial cell types displayed characteristic patterns of substrate preference. The glial cells developed distinctive morphologies on different substrates after incubation for 4 days. Measurements of glial cell migration showed that the substrates did not significantly influence cell proliferation rates. Our results indicate that progenitor cells, oligodendrocytes, type-1 astrocytes, and adult astrocytes possess different compositions of receptors for the adhesion molecules in their environment. Transduction of this substrate-receptor binding signal may induce cytoskeletal alterations that produce the observed morphological differences in glial cells.


Rat primary olfactory neurites are localized in glomeruli and can be regenerated during adulthood. It has not been determined whether the unusual structure or the regenerative capacity of the glomerular system is due to unique growth properties of the olfactory sensory neurons (OSNs), to unique properties of the olfactory bulb (OB) glia that surround the OSNs and glomeruli, or both. However, the following: (i) the regeneration of two types of OB glia, OB astrocytes and ensheathing cells, suggest that the formation of neural circuitry in the OB, as in other areas of the nervous system, is controlled by glial-neuronal interactions. OB astrocytes resemble type-1 astrocytes, and some of the OB astrocytes extend the glomeruli and express the molecules J1 and chondroitin sulfate proteoglycan (CSPG) that have been associated with corticobasal and corticothalamic axons. Ensheathing cells resemble Schwann cells and ensheathe OSN axons from the glial lamellae to the glomeruli. Further, OB astrocyte cell lines with the characteristics of OB glia in the glomeruli-enriching glia promote very low neurite outgrowth by chick retinal ganglion neurons (CRGNE), as expected of glia which form barriers, while other OB astrocyte lines and the high OSN outgrowth, as expected of glia that form axon-growth permissive pathways. In this study, rat OSN neurite outgrowth was tested on both the same cell lines. The results are that neural OB astrocyte lines that express J1 and CSPG support very low OSN outgrowth, while other OB astrocyte lines and ensheathing cell lines support higher outgrowth, and adult ensheathing lines support higher OSN outgrowth than any OB astrocyte lines. Together, the chick and OSN outgrowth results are consistent with the following hypotheses: (1) outgrowth, some OB astrocyte lines may form corticobasal axons that delineate the glomeruli, while ensheathing cells guide the axons to the glomeruli, and (2) during adulthood, ensheathing cells and perhaps also some of the OB glia may permit OSN axon growth, allowing formation of the glomerular synapses.

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405.3 The NG2 proteoglycan inhibits neurite outgrowth from cerebellar granule cells in vitro. M. C. Lemos, Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

The NG2 antigen is a high molecular weight, chondroitin-sulfate proteoglycan. As a cell surface marker in vivo and identifies a population of developing glial cells in vivo, some of which differentiate into oligodendrocytes. Proteoglycans can play important roles in the regulation of axon elongation and provide a physical barrier to glial-restricted affinities of NG2 to either support or inhibit neurite elongation in vitro.

Tissue culture surfaces were coated with poly-L-lysine followed by either laminin alone (10μg/cm²) or laminin mixed with NG2 (5μg/cm²). Ceruleal granule neurons were isolated from postnatal day 5 and 6 rat pups on discontinuous Percoll gradients and seeded onto these surfaces in media supplemented with bFGF. After 24h, the cultures were fixed and the extent of cell attachment and neurite outgrowth quantified. The neurons attached equally well to both substrates, however, 66% of the cells extended neurites on laminin, while only 21% of the cells grew processes on the laminin plus NG2 coated surface. The extent of neurite outgrowth also differed between the two types of surfaces. On laminin, the mean neurite length was 92.5±11.4 microns whereas on laminin plus NG2, mean neurite length was 54±12.2 microns. Digestion of NG2 with chondroitin ABC did not remove the growth-inhibitory activity. When the laminin concentration was increased to 10μg/cm², NG2 no longer inhibited neurite outgrowth. The glycosaminoglycans, chondroitin sulfate and hyaluronic acid, each inhibited neurite outgrowth when used at 15g/cm² in this assay. These data suggest that NG2 is one of a number of proteoglycans that can inhibit neurite outgrowth. Calls that carry the NG2 proteoglycan on their surfaces within developing neural tissues may provide an unfavorable substrate for axon elongation.

405.5 Differential effects of median and lateral mesencephalic astrocytes on neuritogenesis. L.A. Cavalcante, V. Moura-Retei, J. Garcia, and E.L. Carlesso, Instituto de Fisiologia e Patologia, IPFBA, 21241 Rio de Janeiro, RJ, Brazil.

Sulcal glia in median and lateral sectors of the mesencephalon are heterogeneous in neurite response (Barradan et al., Glia 2:103, 1989). In this study, we have tested for regional differences in mesencephalic astrocytes on neuritogenesis. Dissociated cells from either median or lateral sectors of mouse embryo mesencephalic (MMG or LMG) for 2 days and co-cultured with an anti-MAP2 antibodies, (2) cultured onto poly-L-lysine in serum-free medium with addition of conditioned medium from MG or LMG. Both MMG and LMG on MMG grow long and varicose neurites whereas both MMG and MMG on MMG tend to aggregate and show extensive neurites. These features are also mimicked by conditioned medium from LMG or MMG. These results suggest (1) permissiveness vs. non-permissiveness of LMG vs. MMG on neurite growth, respectively; (2) mediation of effects, at least, partially by soluble factors. (Support: CNPq, FINEP, CEPID/GOBI)

405.6 Cultured ensheathing glia from adult rat olfactory bulb enfold olfactory neurites. A Ramón-Cueto, J. Pérez, P. Bovolenta* and M. Nieto-Sampedro, Cajal Institute, 37 Doctor Arce, 28042 Madrid, Spain.

Ensheathing glia of the olfactory bulb is a type of macroglia exclusively present in the olfactory bulb. To gain insight into the mechanisms underlying axonal regeneration in the olfactory bulb, we have studied ensheathing glia and their interaction with olfactory neurites in culture at the ultrastructural level. Three morphologically and ultrastructurally different cell types were identified in secondary cultures from adult rat olfactory bulbs. One of these cells is macrophage-like and showed a cell body with a round nucleus and a prominent nucleolus. The processes of these glial cells spread over the surface of the explant, which suggests that they are ensheathing glial cells. The other cells showed a neurofilamentous arrangement and were shown to be ensheathing glial cells. To examine the role of ensheathing glia in vivo, we used a lesion model of the olfactory bulb and found that ensheathing glia are present in the regenerating axons. These results suggest that ensheathing glia play a role in the regeneration of axons in the olfactory bulb, which is consistent with the presence of ensheathing glia in vivo.
405.9 GROWTH FACTOR INDUCED PHENOTYPIC CHANGES IN CULTURED SCHWANN CELLS INCLUDE ALTERED GAP JUNCTIONAL CONDUCTANCE. K.J. Chaudhuri, M. Chanson, D.C. Spray, J.A. Kessler. Albert Einstein College of Medicine, Bronx, New York 10461.

Schwann cell proliferation, morphology, and gene expression are altered after nerve injury. These changes are reproduced in vitro by agents which elevate intracellular levels of cAMP and by growth factors which are released after nerve damage. We have examined changes in gap junctional conductance in cultured Schwann cell pairs which occurred in association with other phenotypic changes. Antibody directed to reticulon (F2:~60kDa) and the perytanatory extract (BPE:~100kDa), the cells became more spindle shaped, and cell proliferation and low affinity nerve growth factor receptor (NGF-R) expression were increased. Gap junctional conductance was significantly elevated from 0.05±0.01(SEM, n=27) before treatment to 1.23±0.24(SEM, n=19) (p<0.01). By contrast, after exposure to transforming growth factor beta, (TGF-β, 10ng/ml) cells displayed a less flatten, multipolar morphology cell proliferation was not increased and NGF-R expression was decreased. Further, compared to control values, TGF-β significantly reduced cell coupling in a time dependent manner; after 48 hours of treatment, cell coupling was decreased to 20% of the control value. The results indicate that changes in cell coupling resulting from these treatments reflected the number but not the type of channels that were expressed. Electrical or chemical coupling may help to coordinate Schwann cell responses to injury and other stimuli. These observations indicate that the strength of intercellular communication between Schwann cells changes in response to alterations in the cellular milieu providing a possible mechanism for modulating functional interactions between Schwann cells and their environment.


Microglial responses in the rat spinal cord following a hemisection at T0 were examined at levels rostral and caudal to the lesion. Microglia were visualized histochemically using the Griffonia simplicifolia B-isolectin (USA). Expression of interleukin-1 alpha (IL-1 alpha) and tissue factor (TF) was determined in the central nervous system (CNS) using in situ hybridization with mxiRNA-GFAP staining. Lectin staining showed two distinct patterns of the microglial response. First, activated microglia appeared 1 day post-injury (DPI) and were widespread. This reaction was both the white and grey matter. A second pattern of microglial activation occurred 3-4 DPI in the grey matter (C4-C5, T4-T5, T12 and L1) to the level of the lesion. Activated microglia remained prominent for up to 2 weeks in the white matter and 4 weeks in the spinal gray zones. The second pattern of microglial activation was confined to the area of fiber tracts degeneration. The onset of this response was as early as 3 DPI and lasted as long as 2 months. In the dorsal funiculus rostral to the level of the lesion the microglial reaction was confined to the fasciculus gracilis (FG) of the lesion side involving without the adjacent corticospinal tract (CST). Caudally, the response was confined to the degenerating CST without involvement of the FG. By 5 days post-injury, a subpopulation of activated microglial cells showed expression of IL-1 alpha. The localized activation of microglia in regions of degenerating white matter (i.e. FG rostral to the lesion) and not in adjacent, intact regions (i.e. CST rostral to the lesion) suggests a non-directed triggering mechanism for the initiation of the degeneration-induced microglial response. The astrocytic reaction, on the other hand, showed a delayed onset (5 DPI), mostly in the degenerating fiber tracts, and the reaction was less intense than the microglial response during the early survival time. At longer survival times (>4 weeks), however, microglia and astrocytes displayed a largely parallel response in temporal and spatial distribution patterns. (Supported by NIH P01-NS 27511)

405.11 MICROGLIAL RESPONSES TO NEUROTOXIC ABLATION OF SEROTONERGIC AXON TERMINALS. M.A. Wilson* and M.E. Pollinger, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

Microglial cells are a heterogeneous class of non-neuronal cells in the CNS which respond to neuronal damage. Microglia exhibit a number of characteristic features which are altered in response to CNS injury. "Activated" microglia undergo morphologic changes, up-regulation or novel expression of macrophage/miyocyte antigens, and may proliferate and become phagocytic. The response of microglia varies depending on the nature of the particular injury, e.g., neuronal death vs. axotomy.

We have examined the response of microglial cells in the forebrain to the selective chemical ablation of 5-HT axon terminals induced by p-chloroamphetamine (PCA). Rats were treated with PCA (10 mg/kg, s.c., x2) and sacrificed 3 days, 6 days, 3, 6, or 9 weeks later. The dorsal raphe, a 5-HT source in the forebrain, but spares pretermninal 5-HT axons and cell bodies. Antiserum directed against 6-microglial antigens were utilized as markers for microglial activation in PCA treated rats, untreated controls, and rats with unilateral facial nerve transection. Morphologic changes in microglial processes and increases in staining for two of the antigens were detectable 6 days after PCA treatment. Three weeks after PCA, increased expression of four of the microglial antigens was detected, as well as increased branching of microglial processes and changes in the intracellular distribution of one of the antigens. By 6 weeks after treatment, these alterations were less marked; at nine weeks the microglia in treated animals resembled those in controls.


Recently, it has become clear that proliferation of microglial cells and hypertrophy of astrocytes may be a general component in association with the central processes of peripheral axotomy and sensory ganglion cells. For that purpose, by the use of a computerized scanning system, we have analyzed the time course for the microglial (antibody OX-42) and astroglial (antibodies to GFAP and in situ hybridization for mRNA-GFAP) cell reaction in the L4 dorsal horn, the column of Clarke and the gracile nucleus after sciatic nerve transection as well as after the transection of the trigeminal nucleus after infraorbital nerve transection. In all areas examined the microglial cell reaction started 24 to 48 hours and peaked one to two weeks postoperatively. The astrocytic reaction, on the other hand, showed a gradual decline in the reaction over a period of several months. The astroglial cell reaction seems to parallell the microglial cell reaction. These observations demonstrate that peripheral nerve injury induces a rapid and prominent response among microglial and astroglial cells in all the parallel primary sensory projection areas.

405.13 CALCIUM PERMEABLE CHANNELS IN MICROGLIA. M. Jia, M. X. Li, C. A. Colson, and D. L. Gilbert*. Lab. of Biophysics, NINDS, NIH, Bethesda, MD 20892 and Dept. of Physiology and Biophysics, Georgetown Univ. Med. School, Washington, DC 20007.

Cytosolic calcium is important for the activation of microglia. An influx of calcium through calcium-permeable channels may be a means of increasing cytosolic calcium. To study these channels, whole cell recording and single cell recording experiments were performed on cultured rat microglial cells. The calcium channel (in mM): 145 KCl, 1 CaCl2, 1.8 MgCl2, and 10 HEPES; the patch solution (in mM): 110 BaCl2 and 10 HEPES. Non-inactivating inward currents were observed at hyperpolarized membrane potentials. The single channel conductance was about 8 pS. The open probability was voltage-independent. We also report on a voltage-gated L-type calcium channel utilizing a whole-cell recording mode. The bath solution (in mM): 120 TEA-Cl, 15 CaCl2, 2 MgCl2, 10 TRIS-HEPES; the pipette solution (in mM): 120 CaCl2, 2 MgCl2, 5 EGTA, and 10 EDTA. Small long-lasting inward currents were activated with depolarizing pulses at a holding potential of -40 mV. The currents were enhanced with 1 μM Bay K 8644. Due to a lack of an outward K+ current, a slight activation could result in a sustained membrane depolarization. Surprisingly, at high extracellular potassium on microglial function. Using a cytochrome c reduction assay in the presence and absence of superoxide dismutase (SOD), we have measured the production of oxiradicals by resting and stimulated cultured neonatal rat microglia. In order to maintain a normal ionic strength and osmolarity, potassium was substituted for sodium in all experiments. When potassium concentration was changed from a normal of 5 mM to a high of 55 mM, there was no change in resting superoxide radical ion production. A significant increase was seen, however, when phorbi myristate acetate (PMA) was added to activate the microglia to produce the superoxide radical ion. In the presence of 5 μg/ml PMA, potassium concentrations of 25 and 55 mM initiated a 115 percent and a 127 percent increase, respectively, over the PMA stimulated release in normal potassium. This enhancement was partially blocked by 10 μM spiperone. These data indicate that changes in extracellular potassium at sites of injury may serve to modulate microglial release of oxiradicals.


Microglia are the resident CNS macrophages and are found at sites of trauma in the brain. Since injured cells release potassium into the extracellular fluid, we have examined the effect of high extracellular potassium on microglial function. Using a cytochrome c reduction assay in the presence and absence of superoxide dismutase (SOD), we have measured the production of oxiradicals by resting and stimulated cultured neonatal rat microglia. In order to maintain a normal ionic strength and osmolarity, potassium was substituted for sodium in all experiments. When potassium concentration was changed from a normal of 5 mM to a high of 55 mM, there was no change in resting superoxide radical ion production. A significant increase was seen, however, when phorbi myristate acetate (PMA) was added to activate the microglia to produce the superoxide radical ion. In the presence of 5 μg/ml PMA, potassium concentrations of 25 and 55 mM initiated a 115 percent and a 127 percent increase, respectively, over the PMA stimulated release in normal potassium. This enhancement was partially blocked by 10 μM spiperone. These data indicate that changes in extracellular potassium at sites of injury may serve to modulate microglial release of oxiradicals.
406.1

CHANGES IN LOCOMOTION AND NEUROMUSCULAR DEVELOPMENT IN THE NEONATAL RAT ACCOMPANY SIMULATED WEIGHT-LESSNESS. K.D. Walton1, J. Jacoby2, K. Ko1, S.A. Williams1 and R. Linas2, Depts. Physiology & Biophysics and Ophthalmology, NYU Medical Center, 550 First Ave., NY, NY, USA, 10016

Tail suspension of rat pups postnatal day 8 (P8) to P13 leads to abnormal swimming and wobbling (Walton et al., Neurosci. Soc. Abst. 16, 937, 1991). This study asked if: (1) irreversible deficits in motor skills result from an extended period of susp. and (2) susp. alters hindlimb HL muscle development. Pups were susp. P8-P13, P8-P21, P13-P21, or P13-P31. Each susp. (S) animal had a non-suspended companion (C). Free walking was videotaped with high speed camera (nac, 200 fps) and analyzed with Peak 20 motion analysis software. Swimming was unaffected in pups S on P13. Stepping in all S was slow with hyperextension of foot and ankle joints. Animals taken down on P13 or P21 recovered. Deficits persisted in those S until P31, e.g. in those S until P31, e.g. those S until P31, e.g. those S until P31, e.g. those S until P31.

406.3

A KINEMATIC AND ELECTROMYOGRAPHIC STUDY OF LOCOMOTION IN THE KITTEN. L. Girard1, T. Cabana1, and T. Drew2, Depts. Physiology and Biology, Université de Montréal, Québec, Canada, H3C 3J7.

To better understand the development processes of locomotion, a detailed kinematic and electromyographic (EMG) analysis has been undertaken in the kitten. Eight (8) kittens between the ages of 2 and 3 weeks were chronically implanted for the recording of EMG activity from flexor and extensor muscles of the fore- and hindlimbs. Video recordings were always made simultaneously with the EMG recordings. Most kittens were capable of steady treadmill locomotion at a slow speed (0.1 m s⁻1) at 3 weeks of age, but they had difficulty in supporting their weight, and the hindlimbs were placed in abduction. Analyses of the changes in joint angle, at this age, showed that there was a pronounced yield at the beginning of stance at the ankle and, to a lesser degree, at the elbow. By 6 weeks of age, joint angles resembled those measured in adult cats. Even at 3 weeks of age the EMG recordings resembled closely those seen in the adult with flexor and extensor muscles showing strict alternation, with little evidence of co-contraction. Although at 3 weeks there were some subtle differences in the relative timing of several of the muscles, in particular with respect to flexor muscles of the hindlimb, these disappeared by 6 weeks. These results suggest that kittens are capable of generating a normal pattern of locomotor activity at an early age, and that many of the differences in the locomotor capacities of young kittens, compared to adults, are due to their inability to fully support their weight. Supported by the FCAR and the FRSQ.

406.2

HINDLIMB SUSPENSION IN NEONATAL RATS LEADS TO PERMANENT DEFICITS IN AIR RIGHTING REFLEXES. Jane Skirina1, Kerry D. Walton2, Dan Hillman3 and Rodolfo Linas2, Depts. of Physiology and Biophysics and Otolaryngology, NYU Medical Center, 550 First Ave., NY, NY, USA, 10016

Unloading the hindlimbs (HL) of rats by tail suspension from P6 to P21 leads to reversible slowing of the air righting reflex (ARR) (Walton et al., IBRO Absts. 1991). In this study rats were suspended from P13 to P31 to find if the critical period for development of the ARR was the same as that for other motor behaviors and if extending the suspension period would lead to persistent deficits. The pups were suspended by their tails at 20°, and each suspended (S) animal had a non-suspended companion (C). The ARR, elicited by dropping the animals from 50cm onto a padded surface, was videotaped at 200fps. Righting of the head (HL), forelimbs (FL), and HL were measured. Righting of the HL was slower in S than C pups on P19 (341.8 ± 51 msec compared to 212.3 ± 11.9 msec, *p ≤ 0.004). On P31 the mean S and C HL righting times were significantly different at the p ≤ 0.0001 level (350±1.1 and 22±0.4msec) Righting times for the H and FL were not significantly different in the two groups. The differences in HL righting persisted at P60 indicating the existence of a critical period in the development of the central vestibular system (Dieter's nucleus and the vestibulo-spatial tract) beginning near P14 and ending by P31, a period different than that for swimming, but similar to that for walking. These data suggest the existence of a set of "critical periods" organized in a hierarchical manner corresponding to the acquisition of particular motor skills. Preliminary morphological results indicate that the density of neurotransmitters, specifically glycine, may be decreased in Dieter's nucleus in S animals. Supported by NASA.

406.4


Neonatal nerve injury leads to rapid changes in motoneuron synaptic excitation from afferents in the injured nerve (Navarrete, J. Physiol. 438:220P, '91). Here we have studied the distribution of primary afferent fibres around injured motoneurons using a monoclonal antibody to parvalbumin (PV) which preferentially labels large diameter primary afferent fibres (Zhong et al., J. Comp. Neurol. 302:715,'90). Motoneurons were pre-labelled by injection of fluorescent tracers into ankle flexor muscles at birth (P0). At P2, the common peroneal (CP) or the sciatic nerve was crushed unilaterally. At P7 and P14 spinal cords were processed for PV immunocytochemistry. In some cases, motoneurons were injected intrathecally prior to processing. At 7 and 14 days after sciatic crush, the density of PV staining in the ventral horn was markedly decreased on the lesioned side. After CP crush, decreased PV staining was largely confined to the area occupied by the CP motoneuron pool. On the injured side, PV immunoreactive fibres were found in close apposition to the soma and dendrites of labelled flexor motoneurons. We are mapping the distribution of PV-immunoreactive fibres on the somatodendritic surface of injured and uninjured motoneurons. These results show that (1) neonatal nerve injury causes a rapid decrease in PV-immunoreactive primary afferent fibres around lesioned flexor motoneurons. This may, in part, be attributed to death of dorsal root ganglion neurons.
406.6

CHRONIC SPINAL GAP TRANSECTION IN CHICK EMBRYOS: A KINETIC ANALYSIS. N.S. Bradley* and S.H. Chambers. School of Physical and Occupational Therapy, McGill Univ., Montreal, QC, Canada H3G 1Y5.

EMG (Bradley and Bekoff, J. Neurobiol. 92) and observation studies (Oppenheim, J. Cogn. Neurosci. 1990) suggested that the degree of motility is altered in the absence of descending neural inputs in chick embryos. Thus, a kinematic study was undertaken to examine these changes as part of a series of studies on embryonic spinal cord regeneration. The interaction of neural and extraneural factors in motor development.

Midthoracic spinal transections were performed on embryonic day 2 (E2) and continuous video recordings (1 hr) were made on embryonic day 9 for each of 9 embryos. Video records of entire movement sequences (40 s) for 2 embryos were computer analyzed at 60 Hz, the data filtered and corrected for movement out of plane, to measure concurrent wing and leg activity and to compare findings to data for 4 control embryos. Preliminary findings indicate that reduced buoyancy does not alter the overall motility. Linear trend analyses for experimental embryos suggest that relative timing of shoulder/elbow (r2=0.51) excursions and hip/knee/ankle (r2=0.43) are similar to controls (r2=0.47;0.44). While reduced buoyancy does not appear to alter primary features of motility, experimental embryos are not posturally displaced during movements or annomorphic contractions and their movements appear more orderly as compared to controls. This work was supported by FCAR and NSERC.

406.7


Previous results have shown that a spinal cord hemisection at C2 causes an increase in the number of multiple synapses (MS) contacting phrenic motoneuron profiles at the C3-C6 levels of the spinal cord. The reactive synaptogenesis is caused by lesioning the descending bulbo-sympathetic respiratory pathways to the phrenic nucleus or by the generalized effects of spinal cord injury (i.e., edema, ischemia, and the interruption of other inputs) in the immediate area of the phrenic nucleus. To evaluate these possibilities, we carried out a hemisection at either C2, C7, or T4 in 3 groups of rats and allowed a survival period of 7 days. The number of terminals forming multiple synapses and the number of synaptic active zones (SAZ) contacting HRP labeled phrenic motoneurons profiles were counted at EM levels in the spinal cord ipsilateral to hemisection. Seven days post hemisection the percent decrease in synaptic active zones was 86% (C2), 90.7% (C7), and 91.6% (T4) as compared to controls (100%). In order to decrease the decrease in SAZ, the number of multiple synapses increased after cervical hemisections (35.5 ± 5.2 (C2), 34.0 ± 7.4 (C7) as compared to controls (28.3 ± 3.3), but the number of multiple synapses decreased following the T4 lesion (22.8 ± 4.4).

These results suggest that the degree of reactive synaptogenesis in the phrenic nucleus is dependent upon the distance of the spinal cord hemisection site from the target neurons.

406.8


Previous studies have demonstrated a rather weak expression of the crossed phrenic reflex (CPR) in spinal hemisection and an immediate contralateral phrenicotomy in young adult rats (Exp. Neurol. 111:244-250, 1991). There is a significant increase in reflex activity, however, if there is a delay following C2 hemisection before contralateral phrenicotomy. The present study was carried out to determine if conditioning lesions at either the C7 or T4 levels of the spinal cord may also influence the CPR when a postlesion delay of seven days is allowed before the reflex is induced. There were 4 groups of rats: a control group (left C2 hemisection and right phrenicotomy with no preoperative delay), and 3 experimental groups each receiving a C2, C7, or T4 hemisection with a 7 day delay before induction of the reflex. Quantitative assessment of the CPR was accomplished by determining the mean integrated area of phrenic nerve compound action potentials under standardized recording conditions. The results showed that the CPR is significantly increased in the C2 hemisection group (97 ± 34mV, P<0.0003), C7 hemisection group (97 ± 14mV, P<0.01), and T4 hemisection group (31 ± 8mV, P<0.04) as compared to reflex activity in the control group (12 ± 2 mV).

These results suggest that a conditioning lesion in the spinal cord causes an increase in CPR activity. Furthermore, the amount of the increase is dependent upon the spinal cord level of the conditioning lesion.

406.9

ULTRASTRUCTURAL CHANGES IN THE RAT PHRENIC NUCLEUS 2 HOURS AFTER SPINAL HEMISECTION. N.A. Sperry and H.G. Goshergian, Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

This study extends our earlier analysis of injury-induced morphological changes in the rat phrenic nucleus (CJN, 284:519,1989) by examining the nucleus at 2 hrs. post-hemisection (p.h.). Phrenic motoneurons were identified at EM levels by horseradish peroxidase labeling. Micrographs were analyzed qualitatively and quantitatively in both normal and spinal hemisected rats. Our results showed a significant increase in the percentage of dendrodendritic appositions from a normal level of 4.73±0.8% to 8.40±5.4% at 2 hrs. p.h. Although there was not a significant increase in the mean percentage of single and multiple synapses at 2 hrs. p.h. (as first seen at 4 hrs. p.h. in our earlier study), a new finding showed a significant increase in the mean lengths of axonemes in a synaptic active zone from normal lengths of 0.37±2.009m and 0.044±0.07m to 0.41±0.10m and 0.51±0.03m respectively. The hemisected rats and synaptic active zones in the phrenic nucleus could enhance synaptic efficacy by increasing contact area, and从而使 movement direction in our spinal cord injury model.

Previous studies demonstrated that one of the main factors of normal formation and development of structure of central nervous system is afterrehabilitation of motor apparatus. In recent studies we used the local stimulation of the peripheral nerve-muscular system and polarization of the motor cortex. Using this data we developed a method of functional biocontrol (FBC) which aims to increase the rehabilitation of motor disfunctions. Application of this method had high clinical efficiency. We observed the reconstruction of movement, normalization of EEG, ENG, reflex responses of spinal motor neurons and normalization of the habituation process on long-term stimulation of sensory systems. We have shown that this method restores the functional asymmetry in the cortex and spinal cord. Using the method of FBC allows to provide rehabilitation and to decrease the period of treatments 1.5-2.5 time.


Despite its wide distribution in the central nervous system, the role of calbindin D-28k (CB), a calcium-binding protein, is still poorly understood. Recently, Laureo et al. (1991) have suggested a possible neuroprotective role for this protein, in the primate model of Parkinson's disease since tyrosine hydroxylase (TH)-positive neurons containing CB seem to be less severely affected by the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) than those who do not contain CB. The aim of this study was to investigate the ontological appearance of CB in neurons of rat substantia nigra and the degree of co-localization of CB with TH-positive cells in vitro. In serotonin, ventral midbrain tissue containing groups A9 and A10 was dissected from rat embryos at 14 days gestation, enzymatically and mechanically dissociated, then plated on 13 mm-culture dishes coated with poly-L-lysine as substrate. These cultures were grown from 1 hour to 10 days before being fixed by cold paraformaldehyde. The culture disks were then immunohistochemically reacted following standard procedures to reveal TH and CB-immunoreactive profiles.

TH-positive neurons are present in all cell cultures. In cultures grown for 1 hour, round to oval cell bodies without neurites display TH-immunoreactivity. Immunoreactive neurites appear in cultures grown 6 hours. The immunoreactive neurites form clusters and have the characteristic shape of dopaminergic neurons, being mostly polygonal and elongated with main processes arising at each pole of the cell body. In contrast, the immunoreactivity for CB appears in cultures grown for 6 days. CB-immunoreactive cells are oval, smaller than the TH-positive neurons and also tend to aggregate in the form of clusters. In double-labelling experiments in vitro, CB-positive cells were clearly distinct from the TH-positive cells. [Supported by FRSQ and MRC and Canadian Network of Centres of Excellence.]

406.13 THE EFFECTS OF NEONATAL CORTICAL OR CEREBELLAR LESIONS ON THE NUMBERS OF CORTICOSPINAL (CS) NEURONS IN ADULT RATS.

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Previously, we showed that 40000 cortical cells have transient spinal axons that are eliminated by the second postnatal week. The present study determined if increased numbers of CS cells could be retained into adulthood when the cortical fields for growing corticofugal axons are modified. Targets available to growing axons were expanded in the spinal cord by the ablation of the opposite cortex. Brain stem targets, such as the basilar pontine nuclei, were removed after cerebellotomy. Aspiration lesions were performed on postnatal day (PND) 1 prior to cortical lesions reaching the mid-cervical (CS) spinal cord or the development of corticopontine projections. The left frontal and parietal cortices or the cerebellum were removed from pups anesthetized by hypothermia. Pups matured to PND 30 when they were re-anesthetized with methohexital. Fast Blue (FB) soaked pledgets were inserted into the CS tract at CS of all animals. Serial section analysis of FB labeled cells indicate that neonatal cortical lesions do not alter the distribution or number labeled CS cells as compared to litter-mate controls. Cerebellotomy, although incomplete in cases, caused qualitative and quantitative decreases in the distribution and number of CS cells that could be labeled. These data indicate that spared spinal target areas do not result in more CS cells. By contrast, the loss of either the brain stem targets (i.e. basilar pons), or the ascending inputs from the cerebellum, resulted in an increased number of labeled CS neurons. Neonatal lesions anterior to or cerebellotomies result in similar alterations in microstimulation maps of evoked forelimb movements from the cerebral motor cortex. However, the present study indicates the substrate for these alterations in stimulation mapping are different. (Grant support by OU BRSG funds (DOC) & NIH NS01046 (DOC)).

407.1 REGENERATING FROG OPTIC NERVE AXONS: DO GLIA GUIDE THE GROWING TIPS. P.M. Orkand* and R.E. Blanco. Inst. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, PR 00961

Unlike those of mammals, injured optic nerves of frogs can regenerate and eventually form functional connections. Since frog retinal ganglion cell (RGC) axons are slow to degenerate after separation from their cell bodies, the regenerating tips may follow the course of the degenerating axons after a crush injury. In preliminary experiments aimed at studying the relationship of ganglion cells with regenerating axons in the absence of the degenerating ones, we looked at the structure of the proximal tip of the optic nerve 1-2 weeks following transection and deflection of the distal stump.

At early time periods, there is macrophage activity at the cut end, and gial cells are phagocytosing debris of retrograde degeneration in the nerve stump, where regenerating axons begin to appear. After 4 weeks, bundles of positive regenerating fibers lie along phagocytic cells and astrocytes that appear to have migrated into the cut end. Their identification will be confirmed by HRP fillings of RGCs.

Supported by NIH NS07464 and NSF EPSCOR II.

407.2 LEPTOMENIGEAL CELLS CAN INDUCE CHANGES IN ASTROCYTES IN VITRO. R. Moss and S. David*. Centre for Research in Neuroscience, Montreal General Hospital Research Institute and McGill University, Montreal, Canada.

Leptomeningeal (LM) cells rapidly migrate into penetrating wounds of the CNS. Astrocytes in the parenchyma of the CNS that interface with these LM cells transform to form the glia limitans. We have used an in vitro approach to study whether direct interactions with LM cells induce morphological and functional changes in astrocytes.

DiI labeled astrocytes purified from the neonatal rat cerebral cortex were plated onto monocultures of LM cells, astrocytes or polylysine coated glass coverslips. 16-20 hours later the cultures were fixed and the size of labeled astrocytes determined using an IBAS image analysis system. Astrocytes were smaller in size when plated on LM as compared to astrocytes or polylysine. When neurite outgrowth on these astrocytes was assessed by plateing purified cells on either glial cells, there was a 40% reduction in the ability of the astrocytes plated on LM to support neurite growth, compared to astrocytes plated on astrocytes monolayer. These results suggest that leptomeningeal cells can induce both morphological and functional changes in astrocytes, and may account for some of the astrocytic changes at the site of CNS lesions. (Supported by MRC, RHRMSL, FRSQ and PCAN)
407.3


We have developed a model to promote axonal regeneration in the unilateraly injured rat spinal cord (Adv in Neurosurg 3; 41- 44, 1991). Central neurons in the injured rat (female Wistar rat, 90-190 g body weight) spinal cord showed regrowth and remyelination in the autografted PNS bridge of peripheral nerve. HRP study demonstrated that the regenerant and remyelinated axons originated from the neurites of the spinal cord. In this paper, we examined the relationship between astroglia and regenerating nerve fibers in this model. The examination of the graft spinal cord interface revealed that GFA-P-positivie zone did not inhibit the regrowth of CNS axon into the graft. The A2B5 antibody-positive cells were less continuous developing a use of the spinal cord, whereas glial processes at the anotranlateral column located far from the grafted-inset side were clearly stained with the A2B5 antibody. Electron microscopically, in the graft close to the anotransplantation, a network of astrocytes was attached to the surface of basal laminae. Remyelination of regenerated axons was also observed in the spina cord of normal rats. The formation of a fibrous plate among the cells of the interstitial space were easily seen especially along the perivascular area. Astrocytic processes were closely apposed to the regenerant axons with or without giall basal laminae. These results indicate that astrocytic action does not impede the regeneration, rather astrocytes, especially type-1, might support the regeneration of CNS nerve fibers in the milieu of this model.

407.5

INTERACTION OF CENTRAL AXONS WITH SCHWANN CELLS OF PERIPHERAL NERVOUS TISSUE. W. K.D., CHAI, P. DOCKER, K.-F. SO* and K.-C. LAM, Dept. of Anatomy, University of Hong Kong, 5 Tsim Sha Tsui, Pokfulam, Hong Kong.

Optic nerve of 50 rat aged 6 weeks was cut intra-orbitally and replaced by a sciatric nerve graft. After 9 months, the graft was re-operated on and the regenerating optic axons were processed for electron microscopic examination. Various regenerative measurements were made on the myelinated fibers of the regenerating (n=6), age-matched normal optic (n=6) and sciatric (n=6) nerves. The relationship between myelin and axon was examined by plotting the myelin area against the axon area (based on perimeter). It was found that the mean slope of linear regression line for the regenerating group (0.54± 0.045) showed a closer resemblance to that of the optic (0.640±0.024) rather than to the sciatric group (1.29±0.081). The mean g-ratio (axon diameter/Fiber diameter), which indicates the relative sheathe thickness, was different among the three groups. However, there is a tendency for the regenerating group to overlap with the optic group (eg. 165, 64% and 98% of sciatric, regenerating and optic fibers respectively have a g-ratio above 0.68).

Thus, central axons regenerating in peripheral nerve environment seem to be able to alter the myelin production of the Schwann cells in such a way that it supports the idea of axon-dependence in the process of myelination.

407.7


Previous studies have demonstrated that a transected embryonic chick spinal cord loses its capability for axonal repair and functional recovery on embryonic day (E) 13. The onset of spinal cord myelinogenesis is coincident with this transition from permissive to restrictive repair period. To assess a possible inhibitory role of myelin in regeneration, we have delayed the onset of myelination (dysemelination). In such an myelinated embryonic cord, the subsequent assessment of spinal cord repair after a restrictive period transection (e.g. from E13) would serve as a test of whether myelin inhibits or permits axonal growth and functional repair. On E9-E12, direct injection into the thoracic spinal cord of a galactocerebroside monoclonal antibody with a source of spinal cord remyelination resulted in dysemelination until E17, as determined by myelin basic protein immunocytochemistry. A subsequent thoracic cord transection as late as E15 (i.e. during the normal restrictive period for repair) resulted in neurological repair and functional recovery (normally myelinated embryos showed no anatomical repair or functional recovery after an E15 transection). Retrograde labelling of brainstem regions, after regeneration, indicated that anatomical repair of brainstem-spinal projections in dysemelinated, transected chicks was indistinguishable from unoperated control animals. Behavioral (open field) as well as indistinguishable from unoperated control animals. This indicates that the myelination process is inhibitory to the repair of transected spinal cord in embryonic chick (supported by Canadian NCE for Neural Regeneration and Functional Recovery).

407.4

THE FUNCTIONAL PROPERTIES OF PURIFIED POPULATIONS OF CULTURED HUMAN SCHWANN CELLS STUDIED IN VIVO. A.D.O. Levie*, V. Guevadr, P. Aebersco, B.P. Bunge*. The Miami Project and Dept. of Neurosurgical Sciences, University of Miami School of Medicine, FL 33143 and Research, Provence, RI 02912.

Methods are now available to purify populations of Schwann cells (SCs) derived from adult human autographs. Two populations are seeded with 30:70 solution of Matrigel®/DMED (MD) with or without human SCs at a density of 80 million cells/ml. Channels were implanted within 8 mm gap of the transected sciatric nerve of male rats for a period of 6 weeks. The number of regenerated axons and cable surface area was uniformly greater in channels seeded with human SCs when compared to channels containing MD only. Survival of the transplanted human Schwann cells was established by demonstrating the regeneration process within 5 days in culture and immune staining for primates and rat specific nerve growth factor receptor and SI10. These results also indicated that the regenerated axons contained a mixture of human and rat (host) SCs. Myelination of regenerating SCs appeared to occur by both donor and host SCs. We are using immune staining for HNK-1, an antigen found in human but not rat myelins, to establish the presence of human myelin segments within the regenerating cable. These studies indicate that purified populations of cultured human SCs survive and enhance axonal regeneration when transplanted into the injured peripheral nervous system of the immune deficient rat. (Supported by NS 19923 NIH/NINDS, Glaxo, Inc., and The Miami Project. Dr. A. Levie is a Fellow of the Medical Research Council of Canada.)

407.6


Semipermeable guidance channels do not support the regeneration of ventral root axons across a large gap in adult rats. The guidance channel's luminal environment was changed by adding transplanted adult rat derived primary Schwann cells (SC) seeded in a laminin-containing gel (LCG) to provide an axonal outgrowth-promoting substrate. Adult Fisher rat-derived SCs were cultured and used based on 10-30% dissociation. Culture purity ranged from 94-46% SCs. Semipermeable channels were seeded with SCs (5-6 x 10^6 cells/ml) in LCG, or the LCG alone, and tested in a 250 mm gap injury model in adult male Fisher rats. Twenty-one rats received SC grafts and 8 rats received LCG grafts. Regeneration was assessed at 4 weeks post-implantation by counting the total number of axons on cross sections at the channel's midpt. Regenerated cables were found in 19 out of 21 of the SC graft recipients (mean total axons = 2630 ± 270), while only 2 of the LCG grafts resulted in cables containing a small number of axons (mean = 302 ± 225). Passage number and SC purity did not significantly affect the total number of axons in the regenerated cables. In order to delineate the role that the grafted SCs play in the regeneration process, a recombinant retrovirus encoding human placentual alkaline phosphatase (AP) was used to generate a population of AP-expressing primary adult innervated rat SC cultures which were then tested in the L5 ventral root model. Histochemical analysis of the regenerated cables at 4 weeks revealed the presence of AP-positive cells and demonstrated the ability of transplanted SCs to myelinate and ensheath host axons.

407.8


Previous studies in our lab have established that embryonic chick loses its capacity for substantial spinal cord regeneration among embryonic day (E) 13. Correlating with this transition, we have examined changes in protein expression using high resolution 2D gel electrophoresis (1992). We have identified sets of proteins; early neural proteins (ENPs) which are continuously present during spinal cord development (E10-E17) then decrease to relatively lower levels, and late neural proteins (LNNPs) which are only expressed at high levels after E13. Some of these ENPs and LNNPs may play direct or indirect roles in establishing permissive or non-permissive environments for the outgrowth of axonemuratorized fibers. Spinal cord myelin also appears at E13 and may inhibit axonal regeneration. We have also examined changes in the levels of these ENPs and LNNPs after 1) spinal cord injury (transsection) and 2) delaying the onset of myelination (dysemelination).

Embryos were subjected to complete spinal cord transections during the permissive (E10) and restrictive (E15) periods for axonal repair. Expression profiles of the ENPs and LNNPs changed between the E10 and E15 periods, at increments of 2 days, and compared with the up-regulated developmental expression profiles. Two of the ENPs appear to be up-regulated in response to spinal cord injury. In dysemelinated animals, the higher expression of some LNNPs was delayed until well after E13, suggesting that they are myelin associated. These preliminary studies have used high resolution silver staining to visualize the proteins. Currently, we are attempting to confirm and extend on these results with isotopic labelling techniques. (Supported by the MRC of Canada).
RESPONSES OF MACROPHAGES IN DORSAL ROOT GANGLION TO PERNERAL NERVE INJURY. X. Xu and P.M. Richards.. Div. of Neurosurgery, Montreal Gen. Hosp. & McGill University, H3C 3A4. The signals following peripheral nerve injury that induce a regenerative programme in the nerve cell body may involve cells in the vicinity of nerve cell bodies. Previously, the recruitment of inflammatory cells to a dorsal root ganglion was shown to correlate with macrophage regeneration in the corresponding dorsal root. More recently, immunohistochemistry with monoclonal antibodies to a cytotoxic T cell/macrophage marker (ED1), the lA antigen (OX-6), and the complement receptor (OX-42) were performed on cryostat sections of fifth lumbar dorsal root ganglia 0-16 days after sciatic nerve transaction. With all three antibodies, immunopositive cells were present in normal ganglia, the total number being several thousand per ganglion. Immunopositive cells had increased in number by 4 days after injury and were 1-8 times more numerous than normal after 16 days. The higher counts could result from recruitment of macrophages and/or increased immunoreactivity of resident macrophages. The modest inflammatory response surrounding sensory nerve cell bodies is a potential stimulus to regeneration of their axons.

SEAL FORMATION IN TWO TRANSPLANTED GIANT AXONS SUGGESTS TWO MODELS OF SEALING. Todd L. Krause, Harvey M. Fishman, Martin L. Ballinger, and George D. Bittner. University of Texas at Austin, 78712 and University of Texas Medical Branch at Galveston, 77555. Sealed axons could seal according to the following two models: MODEL 1. Constriction at a cut end reduces the opening and increases the resistance of the conducting path between axoplasm and external solution; sealing is accomplished if axoplasmic fusion occurs. MODEL 2. Injury-induced vesicles migrate to the cut end, occlude, and subsequently fuse with each other and the axolemma to form a complete seal. Previous studies have not distinguished between these two models; in fact, we now show they have not determined whether or not sealing occurs. To distinguish between these two models and to determine whether complete sealing has occurred, we have used several electrophysiological measures (membrane potential, complex impedance, and injury current) together with video-enhanced, light, and electron microscopic observations to assess sealing in giant (80-500 µm diameter) axons of squid (Architeuthis dux) and Sepia officinalis (cuttlefish). We found recovery of resting potential and input resistance (Ri) in both axons. However, injury current (i) persisted at a substantial level relative to precut level (background) in squid giant axon (GA) for 2.5 hr following transaction whereas i in earworm medial giant axon (MA) decayed to background within 20 min. Further, the decay of i to background in MAs correlated with the time course of Ri recovery. Morphological observations of the cut end, together with the electrical data, indicate that MAs seal completely by vesicle plugging of a slightly constricted end whereas squid GA constrict at the cut end to produce an increase in the resistance but the end remains open. Support: CNR (N0014-90-J-113), TX ATP, and NSF (ECR-891-5179).

PROTEINS FROM SCIASTIC NERVE CONTAIN A SIGNAL PEPTIDE THAT MEDIATES TRANSPORT THROUGH THE AXON TO THE CELL BODY AND INTO THE NUCLEUS. C. C. Huang, R. Schmid, R. T. Anrub, and C. Nolte. Anatomy and Cell Biology, Columbia University, New York, NY 10032. Axons undergo structural changes in response to environmental clues or injury. While these changes require transcription, it is not clear how the needs of the periphery are communicated, often over long distances, to the nucleus. Using organotypic culture systems, we discovered a pathway in Aplysia neurons that conveys protein precursors from the axon periphery to the nucleus (L. U. Nurney, in press). Access to the transport system depends upon a signal peptide (sp) H-Prx-Lys-Lys-Lys Arg-Lys-COOH. To obtain a probe that might be used to identify neuronal signal molecules that use this pathway, we coupled sp to keyhole limpet hemocyanin and injected the conjugate into rabbit. A polyclonal antiserum was then affinity purified using immobilized sp. When proteins extracted from sciatic nerves were separated by SDS-PAGE, a soluble 35 Kd peptide (sp55) was the major constituent detected by the antibody. Interestingly, when these soluble proteins were subjected to gel filtration on Sepharose S-200, the major immunoreactive fractions, detected by dot-blot assay, were found in a high molecular weight (105 Kd) fraction. However, Western blots showed that this fraction contains sp53. To look for transport, the 105 Kd proteins were coupled to rhodamine (TRITC) and microinjected into the Aplysia neuronal cell body. After 1 day, TRITC-protein was found in the nucleus. In contrast, sp53 extracted from a gel after SDS-PAGE was not transported after injection. These results indicate that endogenous sp-containing proteins exist in mammalian nerve, and suggest that these proteins must be in their native form, or complexed to a carrier molecule, in order to be transported.

RESPONSES OF RETINAL GANGLION CELLS TO AXOTOMY: DIFFERENCES WITH AND SIMILARITIES TO PERIPHERAL NEURONS. M. R. Weiler and U. Vaiyda. Nerve Regulation Research Laboratory, Dept. of Veterans Affairs Med. Center, Northport, N.Y. 11766 and Dept. of Neurology, SUNY, Stony Brook, N.Y. 11794. The reaction of retinal ganglion cells to intraocular crush lesions of the optic nerve was investigated over the first two weeks postlesion using the binding of [3H]Actinomycin D (Act. D) to nuclei of neurons in tissue sections. Changes in nuclear binding of Act. D reflect structural changes in chromatin associated with transcriptional activity. Adult male Wistar rats received intraocular crush lesions of the optic nerve and were utilized at 1, 2, 3, 4, 5, 7, 8, 9, 11, and 14 days after injury. Binding of nuclei for Act. D decreased sharply at one day after injury followed by a transient increase to above normal levels at days 2-3. The remaining pattern of response was characterized by both normal binding 4-7, 9 and 14 days, separated by increases to normal levels at 8 and 11 days after injury. Alterations of Act.D binding were also observed in the contralateral eye. Normal binding was observed at 3, 4 and 9 days and a primary increase in binding at 7 days after the operation. Except for magnitude, the basic pattern of response of surviving central and peripheral neurons to axon injury is similar, but the magnitude of the response is much less in central neurons.

IGNALS THAT INDUCT SPROUTING IN THE CENTRAL NERVOUS SYSTEM: SPROUTING IS DELAYED IN A MUTANT MOUSE EXHIBITING DELAYED WALKERIAN DEGENERATION. D. Stewart. Departments of Neuroscience and Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908. This study evaluates whether sprouting in the CNS is initiated by signals related to the degeneration of presynaptic axons. We evaluate the time course of sprouting of cholinergic septohippocampal fibers after unilateral entorhinal cortex lesions in a substrain of mice carrying a mutation which leads to a substantial delay in the onset of Walkerian degeneration. This is the "Oba" mutation, which has been characterized in detail by Perry and colleagues (Perry, V.H., Lune, E.R. Brown, M.C., Caluacs, S., and Gordon, S. Eur. J. Neurosci., 2: 408-413, 1990). It is thought that axonal degeneration is delayed because the mutation affects a signaling mechanism for macrophage activation. We assessed the time course of Walkerian degeneration after entorhinal cortex lesions using the Fink-Heimer technique. Cholinergic sprouting was evaluated using a histochimical technique for acetyl-cholinesterase (ACHE). In normal control mice, both the time course of Walkerian degeneration, and the time course of cholinergic sprouting after EC lesions occur with a time course that is comparable to that described in rats. Argyrophilic degeneration was prominent by 4 days postlesion, and increases in ACHE staining were well-developed by 8-10 days. In mice carrying the Oba mutation, however, argyrophilic degeneration was not detectable at 4 or 6 days postlesion, began to appear in the dentate gyrus by 8 days postlesion, and did not become prominent until 12 days. Increases in ACHE staining in the molecular layer of the dentate gyrus were not detectable even at 12 days postlesion, but developed gradually after 14 days. These results indicate that the signals which initiate at least one form of CNS sprouting are related to the degeneration of presynaptic axons. Supported by NIH grant NS12533.

THE APPEARANCE OF PRE-MITOTIC MICROGLIA ASSOCIATED WITH AXOTOMIZED MOTOR NEURON PERIKARYA IS INDEPENDENT OF AXON STUMP SIZE. T. G. Crawford M.D.* and J. W. Griffin, M.D. Johns Hopkins University School of Medicine, Baltimore, USA 21205. Following axonal injury tritiated thymidine labeled cells, identified as microglia, appear in the region of the perikaryon. These cells are implicated in the repopulation of the degenerated axon. The number of these cells has been seen following axotomy. We report that the timing and relative peak abundance of these labeled cells does not vary with location of the axonal lesion. At age 3 weeks both tibialis anterior muscles of Sprague-Dawley rats were injected with 1% fast blue. At age 8 weeks the left innervating nerve was severed, either by ventral root section of L4 and L5 (13 mm from the spinal cord) or the peroneal nerve at the level of the fibular head. One, and 4 days later, 4 hours following intraperitoneal injection of 5 pCi/ml tritiated thymidine, the animal was killed and labeled cells were counted, or within one perikaryal diameter to, fluoroescence labeled motor neurons were counted on each side. At day 2 neuron-associated labeled cells were abundant (0.59% neuron-associated axonal microglia, 1.17 neuron for distal axotomy). At 1 and 4 days labeled cells were less profuse, although variable for the proximal axotomy. Labeled cells on the control side were rare at all time points. These findings are consistent with the hypothesis that neuron-associated microglia are regulated by factors emanating from the terminal axon or target muscle.
408.3

COMBINATION OF INTRACELLULAR STAINING OF RETROGRADELY LABELLED NEURONS AND ANTEROGRADE FLUORESCENT TRACING: USE OF THE CONFOCAL LASER SCANNING MICROSCOPE.

C.J. Still and M.D. Cassebaum, Dept. of Anatomy, Univ. of Iowa, Med. College, Iowa, IA 52242.

This report describes a combined retrograde tracing, intracellular injection and anatomical fluorescence labeling method using the application of confocal laser scanning microscopy to simultaneously view the morphology of identified projection neurons and the distribution of retrogradely labeled fibers and terminals to characterize the anatomical relationship between these two elements.

With this approach, the retrograde tracer Fast Blue was injected into the bed nucleus of the stria terminalis (BNST) and anterograde tracer tetramethylrhodamine-conjugated dextran was injected into the insular cortex in adult rats. After one week survival time, the brains were fixed and sectioned on a cryostat. Immunofluorescent labeling of retrogradely labeled neurons identified in the amygdaloid complex on 120 μm thick sections were intracellularly injected with Lucifer Yellow under visual control and analyzed with confocal laser scanning microscopy. The results demonstrate that images from very thin optical sections can clearly show potential synaptic contacts between anterogradely labeled and intracellularly projecting neurons. Stacked images from optical sections show, in very great detail, the morphology of projection neurons in three-dimensions. In comparison with other combinations, the present method provides a more simple and efficient means to trace three successive components of a putative neuron chain.

Supported by NS25139.

408.5

THREE DIMENSIONAL IMAGING OF FACIAL NUCLEUS MOTOR NEURONS USING LASER SCANNING CONFOCAL MICROSCOPY.


The rat eye blink reflex is useful for neurophysiological studies of adaptive gain control. We have been interested in methods that might help elucidate the neurobiology of use-dependent plasticity in this reflex. For circuit tracing, one promising approach involves laser scanning confocal microscopy. In this technique, using a Bio-Rad MRC 600 LSCM system and a long-working distance (500 μm) objective (NA = 1.25).

The lid closure during a reflex blink is generated by a passive downward force plus active contraction of the orbicularis oculi (oo), whose motor neurons are located in the facial motor nucleus (FMN). The oo receives innervation from 2 branches of the facial nerve, the upper zygomatic and the temporal. In vivo injections of tetramethyl rhodamine or fluorescein into these 2 branches resulted in retrograde labeling of motor neurons in the FMN. After fixation, the FMN was transversely sliced (400 μm thick sections), mounted and covered. Optical sections were taken through the FMN at 0.5 - 1.0 μm intervals using a Bio-Rad MRC 600 LSCM system.

A total of 32 sections were analyzed. The data revealed that FMN was significantly larger than with conventional microscopy. Optimal sectioning enabled complete 3D imaging, which is important for cell classification, morphometric analysis, and dual labeling of pre- and postsynaptic elements. We are currently exploring other dyes that may be useful with LSCM in elucidating the reflex circuitry and its sites of modulation. In particular, we are examining projections to the amygdaloid complex, which has been implicated in conditioned potentiation of the eye blink reflex. Supported by an NIH grant (THB) and an NSF fellowship (BFL).

408.6

THREE DIMENSIONAL RECONSTRUCTION OF NEURONS USING CONFOCAL MICROSCOPY AND VOLUME RENDERING.

D. Bel, L. Arends, S.H. Creeds, S.H. Chandler' and N.A. Buchwald and M.S. Lepore, Mental Retardation Research Center and Department of Physiological Science, UCL, Los Angeles, CA 90024.

Our laboratory has been using confocal microscopy to visualize neurons in three-dimensions. Intracellular recordings and dye injection (Lucifer Yellow (LY) or biocytin) were performed in neurons obtained from brain slices of neonate or neonate motor nucleus of pinna. LY-filled cells were examined in whole mounts while biocytin-filled cells were examined at 40X in small sections processed by standard immunofluorescent techniques. Neurons were first optically sectioned with a laser scanning confocal microscope (BioRad MRC600). Sections were obtained at intervals of 1.5-μm (z-axis) with the confocal aperture at its minimal diameter. Dependent upon the numerical aperture and the magnification of the objective, optical section thickness varied from 1-4 μm. Three-dimensional visualization was obtained as a series of optical sections, stereo pairs or volume rendering. Volume rendering to permit three-dimensional imaging was performed with commercially available software (VoxBlast, VaxTec, Inc.) which uses alpha blending to create a three-dimensional projection. The software permits rotation around any axis, changes in the source of illumination and changes in the transparency of the image. Rotation around different axes provides a clearer picture of the three-dimensional characteristics of the neuron. Changes in illumination and transparency allow shadowing and surface landmarks to be visualized. Three-dimensional reconstructions permit perspectives to be reconstructed that were difficult or impossible to view in the serial optical sections or stereo pairs. Supported by USPHS HD 05958.
408.7 **IN-VIVO CONFOCAL MICROSCOPY OF THE RABBIT OPTIC NERVE**

**HEAD** Barry W. Thompson*, Selin Jin Chew, Roger W. Beuerman

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The tandem scanning confocal microscope (TSM) allows repeated, noninvasive examination of living tissue, in real-time, with the advantage of serial optical sections, superior contrast, lateral and vertical resolution. We used a new TSM to examine the optic nerve in the rabbit in two modes: as a scanning confocal microscope and as an ex vivo-imaging technique to investigate the human lamina cribrosa. Using a Noran TSM with a 25 mm water immersion lens, and a 60 mm magnification lens corrected for a cover slip, we examined the retina, optic disc and the optic nerve at the disc. At this magnification of 30X, we were able to ascertain the depth of the optic cup and to precisely identify the axons exiting the optic nerve. Using this TSM, we were able to perform a full thickness examination of the retina and optic nerve and the presence of a glial scar. We are currently working on this technique to investigate the human lamina cribrosa. Using a Noran TSM equipped with a 486 PC, we have been able to perform a full thickness examination of the retina and optic nerve. We conclude that Ca^2+ imaging in acute brain slices is feasible. This system may allow the investigation of intracellular-ion concentration dynamics under various experimental conditions.

408.8 **IN-VIVO MONITORING OF CORNEAL NERVE INJURY BY TANDEM SCANNING CONFOCAL MICROSCOPY**

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With its superior lateral and vertical resolution, the tandem scanning confocal microscope (TSM) allows repeated, noninvasive examination of the cornea in vivo. We used a new TSM to examine the nerves in the rabbit, following two modes of injury (mechanical and laser damage) and the image distributed neural activity in vivo.

408.9 **CONFOCAL FLUORESCENCE IMAGING OF [Ca^{2+}]_i-TRANSIENTS IN ACUTE RAT-BRAIN SLICES USING FLUO-3-AM AND A SMALL VOLUME SUBMERGED CHAMBER SYSTEM.**

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In order to establish a simple system for the imaging of intracellular-ion concentration dynamics in brain slices using fluorescent dyes we developed a small volume slice chamber. The chamber volume is ∼ 0.5 ml, affording rapid (< 5 s) exchange of the bathing solution by small amounts of dye. Imaging was performed with a confocal laser scanning microscope (Bio-Rad MRC 600; 488 nm), equipped with a Zeiss x60 NA 0.75 water immersion objective corrected for a cover slip. Images were obtained with minimal dye concentrations and loading time (1 μM, 15 min). The apparent diffusion coefficient, D*, was measured in cortex (Cserr et al. J. Physiol., 442: 277, 1991) but the 40 under a critical size that lies in the range 10-40 kDa. The diffusion of molecules above a critical size that lies in the range 10-40 kDa was also performed after image capture using a CCD camera. Eximer laser photorefractive keratometry was performed, with the ablation of the anterior 75μm of corneal stroma. The regeneration of epithelial nerve endings was observed with the TSM. Histology, using the gold chloride impregnation technique was used to confirm these observations. The epithelial nerves of human contact lens were compared with those of non-contact lens wearers.

408.10 **THREE-DIMENSIONAL ANALYSIS OF GRANULE NEURONS IN THE RAT DENTATE GYRUS LABELED WITH FLUORESCENT DYES.**

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Efforts to analyze the 3-D structure of fluorescently-labeled neurons have been hindered by the lifespans of the fluorochromes. Dense labels, which are more difficult to use, have been required. Here we report a technique that allows neurons labeled with DiI or Lucifer Yellow (LY) to be analyzed using a computer-microscope system equipped with epi-fluorescence optics. Glucose neurons in 400-μm-thick slices of the dentate gyrus were labeled either by retrograde transport of DiI in fixed tissue (Chabner & Chabner, J. Neurosci. Abstr. 17: 35) or by intracellular injection of LY in vivo or in fixed tissue (Pfau-Borg et al., Neurosci. Lett. 118: 289). To minimize bleaching of the dye, the intensity of the mercury excitation (Nikon H2A or Omega H2B) was decreased using neutral density filters, and the field of view was reduced to compensate for the decrease in fluorescence emission. We used a Hamamatsu C2400-08 camera and a Rolyn KG2 heat filter. We use this system to investigate the reliability of the system for 3-D analysis of fluorescently-labeled neurons. Supported by MABRS and ONR.

408.11 **QUANTITATIVE OPTICAL IMAGING OF THE DIFFUSION OF DYE TRANSPORTERS FROM DIFFERENT MOLECULAR WEIGHTS IN RAT CEREBRAL CORTEX.**

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Many permeable substances have molecular weights of 1 - 100 kDa and is important to know how they diffuse in tissue. We have developed a quantitative optical imaging system and analyzed the diffusion of 3, 10, 40, and 70 kDa dextran to determine molecular weights in the cortex.

Dextran labeled with the fluorescent dye Texas Red (Texas Red, Molecular Probes, OR) were pressure-injected into rat cortical slices maintained in a perfused chamber at 34°C and imaged as they spread in the tissue, using a confocal epifluorescence microscope with a 10X water immersion objective. About 20 images were taken with 2 - 10 second intervals and recorded by a cooled CCD camera (Photometrics, AZ) with 576 x 384 pixels and 14 bits and transferred to a 486 PC. The apparent diffusion coefficients, D, was determined by fitting an integral expression relating the measured 2-dimensional image intensity to the 3-dimensional dextran concentration. Measurements in dilute agarose gel provided a reference value of D. Values of the ratio, (D/D0)^0.5, for the 3 and 10 kDa dextrans were consistent with tortuositites derived from terephthalamide measurement in the range of 1.427 - 2.77, 1991) but the 40 and 70 kDa dextrans showed markedly larger ratios. This suggests that extracellular space may not be uniform but has consistencies that hinder diffusion of molecules that lies in the range 10 - 40 kDa. This implies that the concept of tortuosity, as a simple parameter describing the diffusion properties of the extracellular space, is valid only for molecules below this critical size. Supported by NIH Grant NS 28642.

408.12 **CCD-IMAGING OF VOLTAGE SENSITIVE DYE SIGNALS FROM WHOLE BRAIN OF LOWER VERTEBRATES.**

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We used the fluorescent potassium membrane probe Di-4-ANEPES to image distributed neural activity in vivo and in isolated whole brain preparations of frog, lizard and turtle. These animal models were obtained with a CCD at a rate of 1 Hz and produced to process movies of the spatio-temporal patterns of voltage changes. Small arrays of diodes were used to measure voltage changes with high temporal resolution from selected regions. Simultaneous recording of optical and electrical signals was possible for periods >8 hours without light-induced toxicity.

Optical recordings reliably showed the onset, duration, and propagation of neural responses that were evoked by stimulation of cranial nerves or brainstem structures, by disinhibition with picrotoxin (PTX) and by light or odor stimuli. Optical signals generally differed from field potential recordings by the presence of long lasting slow components, that often persisted many seconds after a stimulus. In contrast, a close correspondence between the time course of optical signals and intracellularly measured voltage was seen suggesting the slow components are not glial in origin. Spontaneous activity induced by PTX originated in brainstem and propagated along several defined paths (e.g. anterior dural telencephalon to thalamus/ hypothalamus). Repeated initiation of these “epileptiform” events from the same sites was observed over the course of several hours. Telencephalon initiated events occurred more frequently and recovered faster than those in diencephalon, tegmentum, cerebellum or brainstem. Odors or olfactory nerve shock produced optically recorded oscillatory activity in olfactory bulb and activity extending into ipsilateral and contralateral caudal telencephalon.

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808.13

SIMULTANEOUS KINETIC IMAGING OF INTRACELLULAR CALCIUM AND pH IN SINGLE MELANOTROPES. Stephen J. Morris*a, Diane B. Boyettφ, and Bibi M. Chromwell*. Div. of Molecular Biology and Biochemistry and TDIV, of Cell Biology and Molecular, Univ. of Missouri-Kansas City, Kansas City, MO 64110

There is a growing interest in the use of multiple fluorescence probes to analyze the relationships between [Ca2+]i and pH. We have designed an epifluorescence video microscope for calcium and four affording high resolution which simultaneously capture all four emission images at 405, 475, 575 and 640 nm from the two ratio dye indo 1 (for [Ca2+]i) and SNARF 1 (for pH) at video rates (SPIE Proc 1428:148,1991).


Popular dyes for measuring intracellular calcium, such as indo 1 and fura 2, have pH-dependent Ke and pH changes in pH, can be misinterpreted as changes in [Ca2+]i.

SNARF sensitivity to pH between 6.5 and 8.0 was unchanged by [Ca2+]i. The indo/Cr3+ Ke, examined at 390 and 37°C, shifted more than twice fold between pH 6.5 and 8.0. Rat primary melanocytes, grown in explant cultures, were double-labelled and Cr3+/pH interactions were examined during exposure to various stimuli. SNARF ratio maps were used to correct the pH-dependent changes in local cell calcium. Under most circumstances, pH correction modified the apparent [Ca2+]i: small changes in [Ca2+]i disappeared or were greatly attenuated and kinetics changed. Cells were later positively identified as melanotropes by immunohistchemistry.

K+-induced depolarization of murine fat produces increases in [Ca2+]i, which were closely coupled to reductions in pH, Chronically applied dopamine agonists suppressed this activity, which returned when the drugs were removed. Cells responded to both N’-isoflurane and subsequent acidification upon NH4Cl withdrawal with calcium transients from intracellular stores. We conclude that the new video microscope will be an invaluable tool for the study of intracellular dynamics.

808.15


Cells and cellular organelles can exist as the network of micros and therefore cannot be encompassed in a single thin section normally examined with conventional electron microscopy. Even with the use of high voltage electron microscopy, section thickness is limited to no more than a few microns. In an effort to overcome these limitations we have been exploring a method of linking serial thick sections by first extracting these three-dimensional information using tilting tomography and then aligning and linking the resulting serial volumes to form a single volume. We have used this method to investigate the 3D structure of the Golgi apparatus in dorsal root ganglion neurons. Briefly, four 2um serial sections were cut from an osmium stained, unperfused dorsal root ganglion (Lindsey et al., Neurosci. 12:3111, 1985). A tomographic reconstruction was created for each section of a single axis tilt series taken through 60um in 2um increments with a JEOL 100S electron microscope operated at 400KV. Alignment of the serial volumes was accomplished by slicing each volume into individual planes orthogonal to the depth dimension and registering the last slice of one volume to the first slice of the next with the aid of fiducial marks. The aligned slices of the 4 volumes were then merged into a single volume. The resulting volume was rendered and examined using the program ANALYZE (Rohrb et al., IEEE Trans Med Image, MI-8:217, 1989). The continuity of components of the Golgi apparatus could be observed through a depth of 10um with spatial resolution near to 100nm. This method appears promising for characterizing the 3D organization of subcellular structures over many micros while maintaining resolution sufficient to discern fine structural detail. While the alignment of the serial volumes appeared accurate, we are currently validating this technique by using a specimen of known 3D geometry. We are also exploring the use of this technique to investigate the continuity of neuronal endomembrane system components in bulging dorsal root ganglion and in cerebellar Purkinje cell dendrites.

808.17


In spite of high resolution obtained by modern imaging techniques it is necessary to supplement these data with histological and histochemical data from the microscopical examination of brain sections. In order to integrate these data we have created a 3-D human-brain-reference atlas. This atlas is based on one parafin-embedded brain (death to fixation interval: 2h), which was serially sectioned vertical to the intercommuncating plane. Cytomorphology and morphometric studies of this brain and correlations with immunohistochemical findings have been published by numerous authors. From 630 sections contournitures representing pit and ventricular surfaces, as well as subcortical nuclei and their subdivisions, were enzymatically cut manually. The contournitures were adjusted into a 3-D metric grid. Points of intersection between the contournitures and the grid were used for cubic spline approximation. The resulting curved panel was extrapolated to be manipulated after estimations of deformation and shrinkage due to tissue preparation. The smoothed contournitures were used for 3-D reconstruction. Contournitures of main thalamic subnuclei have been comparatively compared regarding to the interindividual variability, with those derived from paraffin-embedded brains (applying the same procedure of in-vitro - in-vivo transformation; 25 hemippheres) and MR imaging (18 hemippheres).

808.18


Past methodologies for analyzing cerebral microvascular system have employed single histological sections and stereotactical theory. Such approaches do not yield information on microvascular branching, length, and tortuosity. Since these parameters are suspect we suspect that some of these features vary among brain areas, three-dimensional reconstructions of local microvascular systems were made using serial histological sections and an image analysis system (MCID IM, Imaging Res. Inc) The results indicate that cerebral microvascular systems vary considerably in branching and tortuosity. For example, the capillaries in the hippocampus are fairly straight, long, and arise by simple division of the terminal arterioles, whereas those in the paraventricular nuclei form tortuous networks of many relatively short capillaries. Microvascular system organization, thus, seems to vary among brain areas, possibly in the liver.
**CALCULUM CHANNEL TOXINS II**

409.1

**EFFECTS OF THE CALCIUM CHANNEL BLOCKER OMEGA-CONOTOXIN ON IN VIVO DOPAMINE EFFLUX IN RAT STRIATUM. C.A. Davy, C.D. Blaha* and A.O. Phillips, Dept. Psych., University of British Columbia, Vancouver, BC, Canada, V6T 2Z4.** The effects of omega-conotoxin (CTX, fraction GVI), an N- and L-type voltage-sensitive calcium channel (VSCC) blocker, on basal dopamine efflux was investigated in vivo using electrochemistry (chronamperometry, 1 s potential pulse/30 s). A 30 gauge cannula was positioned 0.5 mm adjacent to a stereotaxic graphite probe. The efflux electrode and was stereotaxically implanted into the dorsomedial striatum of urethane anesthetized rats. Following at least 1 hr of baseline recordings, injection of CTX (0.1, 1 or 10 pmol/ul 4 min) produced a dose-dependent decrease in basal DA efflux. A maximal decrease in DA efflux was achieved within 10 min of injection of 10 pmol CTX. This decrease was followed by a rebound in DA efflux which increased significantly above preinjection values within 1 hr of CTX injection. Additional CTX (10 pmol) injections during this rebound phase produced decreases in DA efflux that were similar in magnitude and time course as the first injection. These results provide some of the first in vivo evidence indicating that both N- and L-type calcium channels are involved in basal DA transmission and suggests that transient inhibition of these channels may lead to increased DA efflux. These findings are in contrast to in vitro studies which demonstrate an irreversible blockade of VSCCs by CTX. The mechanisms responsible for the transient and rebound effects of CTX are unknown at present, but may include a biphasic modulation of VSCCs or increased storage of DA at the presynaptic terminal.

409.2

**NOREPIPHRINE (NE) RELEASE FROM CARDIAC ADRENERGIC NERVE TERMINALS: AGE-RELATED DIFFERENCE IN OMEGA-CONOTOXIN INHIBITION. D.L. Snyder, V.J. Aloyo*, M.D. Johnson, and J. Roberts, Dep. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.** Cardiac sympathetic (SYN) preparations from hearts of 6 and 24 mo old male F344 rats were used to investigate the effect of age on omega-conotoxin (CTX) inhibition of NE release. CTX is a specific N-type Ca**2+** channel blocking agent. SYN were incubated in 3H-NE, placed in a superfusion system, and then perfused for 30 min with various concentrations of CTX. The SYN were then depolarized with a 2 min pulse of buffer at 75 mM K+ (concentration which produces maximum release) or 60 mM K+. The mean fractional release of NE (NE released/NE released+NE released, NE released=NE released after vehicle administration was significantly reduced in 24 mo old rats when compared to 6 mo old rats (N=5, see table). 6 mo old rats were more sensitive to CTX inhibition and showed a greater percent reduction in NE release at each level of CTX. These results suggest that the adrenergic nerve terminals of 6 mo old rats contain more CTX sensitive Ca**2+** channels than at 24 mo age. SYN from 6 mo old hearts contain more CTX binding sites than SYN from 24 mo old hearts. The loss of Ca**2+** channels with age may be responsible for the age-related reduction in NE release.

**Mean fractional NE release (% inhibition by CTX)**

<table>
<thead>
<tr>
<th>CTX</th>
<th>0</th>
<th>10 pmol</th>
<th>100 pmol</th>
<th>1 pmol</th>
<th>10 pmol</th>
<th>100 pmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mo</td>
<td>75 mM K+</td>
<td>4.9</td>
<td>4.9</td>
<td>3.7</td>
<td>3.4</td>
<td>3.3</td>
</tr>
<tr>
<td>24 mo</td>
<td>75 mM K+</td>
<td>4.0</td>
<td>4.0</td>
<td>3.8</td>
<td>3.5</td>
<td>3.6</td>
</tr>
<tr>
<td>6 mo</td>
<td>60 mM K+</td>
<td>4.8</td>
<td>4.0</td>
<td>17.4</td>
<td>2.9</td>
<td>2.4</td>
</tr>
<tr>
<td>24 mo</td>
<td>60 mM K+</td>
<td>4.0</td>
<td>3.2</td>
<td>20.3</td>
<td>3.3</td>
<td>2.7</td>
</tr>
</tbody>
</table>

409.3

**BLOCK OF MAMMALIAN CALCIUM CHANNELS EXPRESSED IN XENopus OOCYTES BY \( \alpha \)-AGATOXINS. A. Kondo, J. D. Mills* and M. E. Adams, Departments of Entomology and Neuroscience, University of California, Riverside, CA 92521.** \( \alpha \)-Agatoxins from Ageleoposis aperta spider venom are potent and selective blockers of voltage-activated calcium channels. We are using these toxins as probes in comparative studies of Ca**2+** channels expressed in Xenopus oocytes. Oocytes injected with mRNA prepared from rat brain, whole brain, and cerebellum displayed voltage activated currents through Ca**2+** channels using Ba**2+** as a charge carrier, TEA, 4-AP, and niflumic acid were used to block potassium and chloride currents. Oocytes expressing mRNA from heart muscle showed Ba**2+** currents with kinetics typical of L-type Ca**2+** channels. Exposure to 100-200 mM \( \alpha \)-AgA-III resulted in 30-90% block of high threshold current, with little or no effect observed on low threshold currents. Expression of mRNA from whole brain resulted in Ba**2+** currents that were blocked at 100-200 mM \( \alpha \)-AgA-II, but only 1-5% by 400 mM \( \alpha \)-AgA-IVA. In contrast, oocytes injected with cerebellar mRNA displayed Ba**2+** currents that were substantially blocked by both \( \alpha \)-AgA-III and \( \alpha \)-AgA-IVA. Based on the selectivity of \( \alpha \)-AgA-IVA, these results suggest that P-type channels are relatively more abundant in the cerebellum than in whole brain. Supported by NIH grant NS24472.

409.4

**A NOVEL PEPTIDE NEUROTOXIN SELECTIVELY BLOCKS MYOCARDIAL L-TYPE CALCIUM CURRENT. J.J. McArdle, Y.-F. Xiao, S.P. Aiken*, L.C. Sellin, J.J. Schmidt, and S.A. Weinstein, Dept. of Pharmacology & Toxicology, New Jersey Medical School (UMDNJ), Newark, NJ 07103-2714, and Dept. Toxicology, USAMRIID, Frederick, MD 21701-5011.** Peptide I (WTX-I) from the venom of Trimeresurus walgeri appears to act presynaptically with synaptic transmission (Pharr & Tox. 1992, in press). In order to explore the interaction of WTX-I with ion channels, we examined its effects on L-type Ca**2+** (\( I_{Ca} \)) and transient K**+** (\( I_{K} \)) currents, as well as Na**+** (\( I_{Na} \)) currents recorded from the atrial myocardial myocytes from the left ventricle of 10-11 week old rats. Ionic currents were recorded with the whole cell configuration of the patch voltage-clamp technique. Bath application of 6 to 20 mM WTX-I had no detectable effect on \( I_{Na} \) or \( I_{Ca} \). In contrast, 2, 4, and 6 M WTX-I reduced \( I_{Ca} \) to 68.4 ± 15.4 % (n=3), 47.7 ± 6.0 % (n=11), and 10.1 ± 10.1 % (3) of the respective control values. This inhibitory effect on \( I_{Ca} \) could not be reversed by applied to 5 mM nifedipine. Recovery occurred after washout of WTX-I containing solution. These findings suggest that WTX-I selectively blocks L-type Ca**2+** channels and that stimulation of \( \beta \)-adrenergic receptor-modulated phosphorylation of this channel may increase the inhibitory effect of WTX-I. Supported by grants from the NIAAA (AA008025) and the American Heart Association (90-G-27).


4.9.10 NEOMYCIN DISPLACEMENT OF \( [125] \) \( \omega \)-CONOTOXIN GVIA-A BINDING IS NOT UNIFORM ACROSS NEUROANATOMICAL REGIONS: EVIDENCE FROM AUTORADIGRAPHIC STUDIES. E. Filloux 1, B. M. Olivera 2, and J.M. McIntosh 3, Dept. Neurol., Pediat., Bio1, and Psychiatry, Univ. Utah, Salt Lake City, UT 84132.

Previous studies have demonstrated that neomycin (NEO) inhibits \( [125] \) \( \omega \)-Conotoxin GVIA (\( \omega \)-CTX) binding to brain membranes suggesting that NEO may block Ca channels. However, we have now characterized several new \( \omega \)-conotoxins which inhibit an expanded subset of mammalian CNS Ca channels. Oligonucleotide probes directed to conserved amino acids of the Ca channel-targeting 

4.9.11 Oligonucleotide probes directed to conserved amino acids of the Ca channel-targeting

4.9.12 The venom of fish-hunting cone snails contains potent peptide inhibitors of presynaptic neuronal calcium channels. However, previously characterized peptides such as \( \omega \)-conotoxin GVIA, target only a subset of mammalian CNS Ca channels. We used a molecular biological approach to derive new \( \omega \)-conotoxins which inhibit an expanded subset of mammalian CNS Ca channels. Oligonucleotide probes directed to conserved amino acids of the Ca channel-targeting peptides were used to identify cDNAs encoding two new Ca channel ligands. cDNA sequencing was used to guide synthesis of peptides MVIC and MVIB which inhibited synaptic currents in Purkinje cells and a subset of \( \omega \)-GVIA-resistant channels in CA1 hippocampal cells are sensitive to \( \omega \)-MVIC. In addition, in several systems, \( \omega \)-MVIC inhibits a component of neurotransmitter release that is resistant to \( \omega \)-GVIA or MVIA (see abstracts by Gaur et al., and Newcomb and Palma). Accordingly, these new peptides are of important new tools for the study of Ca channel subtypes.
409.11
CALCULUM CHANNEL ANTAGONISTS [125I]-Ag-Aga-III A AND [125I]-Ag-Aga-III B: A COMPARISON OF BINDING SITES IN RAT BRAIN. J.M. Mcintosh1, M.E. Adams2, B.M. Olivera3 and P. Filloux3, Departments of Psychiatry1, Biology3, Pediatrics4 and Neurology4, University of Utah, Salt Lake City, UT 84132, and Departments of Enzymology and Neuroscience5, University of California, Riverside, California, 92521.

Specific binding of the calcium channel ligands [125I]-Ag-Aga-III A and [125I]-Ag-Aga-III B to rat brain was performed as described earlier. [125I]-Ag-Aga-III A and [125I]-Ag-Aga-III B were compared autoradiographically using sagittal sections from rat brain. Binding patterns for these two ligands were generally similar, but notable differences were detectable particularly in the cerebellum and hippocampus. Specific [125I]-Ag-Aga-III A binding was greatest in the granule cell layers of the cerebellum and of the dentate gyrus. In contrast, binding of [125I]-Ag-Aga-III B was most intense in the molecular layers of these structures. 250 nM [125I]-Ag-Aga-III A inhibited less than 33% of [125I]-Ag-Aga-III A binding while 40 nM [125I]-Ag-Aga-III B inhibited greater than 92% of [125I]-Ag-Aga-III B binding. The P-type calcium channel antagonist, u-Aga-I-JVA (40 nM) blocked only 11% of [125I]-Ag-Aga-III A binding and 2% of [125I]-Ag-Aga-III B binding. These data suggest that [125I]-Ag-Aga-III binding sites are a subset of [125I]-Ag-Aga-III sites and that the combined use of agonists and conotoxins may be useful for discriminating between calcium channel subtypes.

409.13
([125I]-CONOTOXIN MVIA: A NEW, COMMERCIAL RADILIGAND FOR N-TYPE CALCIUM CHANNEL BINDING IN NEURAL MEMBRANES. J.J. Geer1, S.J. Shohr2, and D.J. Dooley1. Department of Neuroscience, Parke Davis Research, Warner Lambert, Ann Arbor, MI 48105.

The conotoxins GIVA and MVIA, isolated from the marine snails Conus geographus and Conus magus, are important tools for identifying and blocking N-type calcium channels in neuronal preparations. Although [125I]-GIVA is commercially available, its irreversible binding characteristics make true competitive binding studies difficult. We recently tested an experimental preparation of [125I]-MVIA (Amersham) and compared this ligand to [125I]-GIVA. We report here the methods for and characteristics of [125I]-MVIA binding. Synthesized MVIA (Peninsula) was iodinated by Amersham using an enzymatic method. Specific activity of the ligand was ~2000 Ci/mmol. Binding of [125I]-MVIA to crude rat neocortical membranes was carried out at 37°C in 0.5 ml buffer (50 mM HEPES-NaOH, 0.1% BSA, pH 7.4) for 30 minutes. Unbound ligand was removed by filtration through G-75 columns presoaked in 0.1% polyethyleneimine (PEI), followed by two washes with buffer supplemented with 0.2 M NaCl. Saturation analysis gave a Kd of 6 x 10^-14 M and a Bmax of 2 x 10^11 M. Dissociation rates were calculated from data obtained from 10-100 nM protein using [125I]-MVIA at 3 A. These results were comparable to those obtained with [125I]-GIVA (Kd = 0.6 pm; Bmax = 995 fmol/mg protein; 23°C). The association kinetics of both [125I]-MVIA and [125I]-GIVA were rapid (t1/2 = 2 min, 37°C at 3 A; GIVA, t1/2 = 4 min, 23°C). However, the dissociation rates were markedly different. [125I]-GIVA was readily dissociated by excess cold ligand (Bmax = 3 min). [125I]-MVIA was not significantly displaced by cold GIVA in 4 hours. Commercial availability of this highly-affinity, reversible radioligand will allow for direct competitive analyses of novel N-type calcium channel modulators.

409.15
Components of the electrically evoked increase in intracellular calcium in chick dorsal root ganglion and sympathetic ganglion neurons. Y. Zhao1 and W.D. Brenbon2. Department of Physiology, University of Minnesota, Minneapolis, Minnesota, MN 55455.

We measured changes in Fluor-3 fluorescence as an indicator of changes in intracellular calcium in primary cultures of chick DRG and sympathetic ganglion neurons. Ca2+ responses were evoked via trains of electrical stimuli delivered through the recording chamber. A 1-2 sec train at 10 Hz produced a rapid and reversible rise in internal Ca2+ in both DRG and sympathetic ganglion cells. The response was prevented by removal of external Ca2+ and by 300 μM 3,4-dichloroisocyanuric acid. This indicates that it is dependent on calcium influx and on the generation of sodium dependent action potentials. In DRG cells 89% (5±2%; n=15) of the Ca2+ response was prevented by brief incubation with omega Conotoxin. The block appeared saturated at a 1 μm solution. 10 μM Nifedipine blocked 138% (4±2%; n=23) of the [Ca2+]i increase induced by 3,4-dichloroisocyanuric acid. These data suggest that the calcium influx in chick DRG cells under these conditions is largely through N-type channels. Type channel appears to play a very minor role, since even relatively high concentrations of N-type channel blockers did not completely block the Ca2+ increase. These data appear to conflict with recent voltage clamp studies suggesting a significant role for T currents during the action potential. In sympathetic cells, Conotoxin blocked considerably less of the Ca2+ rise (63% ±4%; n=7). Very preliminary data suggest that Nifedipine and Amiloride may each block more of the Ca2+ rise in sympathetic cells relative to their effects in DRG cells. (Supported by NHlR T01 GM42829. We particularly thank Than for valuable advice and discussion.)

409.16
Toxins in Pleocyclus spider venom are potent blockers of vertebrate calcium channels. L.A. Newman, Y. Zhao, M.S. Rudnick, and W.D. Brenbon2. Dept of Physiology, University of Minnesota, Minneapolis, MN 55455.

We prepared a partially purified fraction of Pleocyclus trisius spider venom for assays on vertebrate calcium channel preparations. Size exclusion on Sephadex G50 and step elution from C18 with 30% and 60% acetonitrile (0.1% TFA) yields a relatively hydrophobic fraction containing peptides with masses of a few thousand daltons. This fraction has been found in various previous assays to be primarily inhibitory. This inhibitory fraction represents approximately a hundred fold purification from crude venom based on the mass, but it still contains many different peaks when analyzed on reverse phase by gradient elution. We assayed this fraction for activity in blocking electrically stimulated rises in internal Ca2+ in primary cultures of chick dorsal root ganglion (DRG) and sympathetic ganglion neurons. The change in intracellular Ca2+ induced by a train of brief electric voltage pulses was measured as an increase in the fluorescence of the Ca2+-indicated Fluor-3. The inhibitory fraction of the venom blocked 87% (5±8%; n=10) of the increase in fluorescence in DRG cells, and 64% (5±0%; n=15) of the increase in sympathetic ganglion cells. The block saturated at a 1:1000 or greater dilution of toxin relative to crude venom and was not reversible during the time course of the experiments. This toxin activity is similar to omega Conotoxin and likely represents a potent block at least of N-type calcium channels. Preliminary voltage clamp data on these toxins suggest that this fraction blocks Ca2+ currents. Purification and analysis of specific toxin components is in progress. (Supported by NHlR T01 GM42829.)

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409.12
A POLYPEPTIDE FRACTION ISOLATED FROM CONUS MAGUS VENOM EXHIBITS NOVEL CALCIUM CHANNEL BLOCKING ACTIVITY. PREPARATIONS. S.J. Stroeh4, A.B. Giordano, R.R. Naughton, J.M. McIntosh2, D.J. Dooley1, B.M. Olivera3, and D.A. Downs1. Departments of Neuroscience1 and Chemistry, Parke Davis Research, Warner Lambert, and Departments of Psychiatry2 and Biology3, University of Utah, Salt Lake City, UT 84112.

Natural peptides purified from the venom of numerous poisonous organisms have become important tools for understanding neuronal ion channels. We have isolated two polypeptides from venom of the marine small Conus magus which interact with L-type Ca2+ channels. An aqueous extract from crude venom was tested in a [3H]-ipisodine binding assay to assess L-type Ca2+ channel affinity. The C. magus extract was at least ten times more effective at inhibiting [3H]-ipisodine binding to rat neocortical membranes than the extract from other Conus species (relative IC50 = 1/400,000 dilution). We used two reverse phase chromatography steps to isolate the active component, in each step, the inhibitory activity altered as a single peak (absorbance at 214 nm), resulting in a ~300-fold purification of the material. This material inhibited [3H]-ipisodine binding to both neocortical and crude skeletal muscle membranes. Intracerebroventricular injection of this fraction into mice resulted in anchored back and extended extremities, an effect similar to that seen with the L-type Ca2+ channel activator Bay K 8644. This fraction also blocked K+-induced Ca2+ flux into rat brain synaptosomes, indicating a functional interaction with presynaptic Ca2+ channels. When the fraction was further purified using reverse phase chromato-
409.17  

NEURAX Corporation, Menlo Park, CA 94025.

We have synthesized the conceptephide designated SNX-230 corresponding to a novel a-like conceptephide, M-VIIIC, predicted from a cDNA sequence obtained from a Cowan magus venom gland expression library (see abstract by Hillyard, et al.,). SNX-230 potently (20pm IC50) inhibits about 50% of release of radiolabeled noradrenaline (NE) from K+-depolarized rat hippocampal slices. It inhibits the remaining 50% with much lower potency (65mM IC50). In contrast, c-conceptephides SNX-111 (synthetic M-VIIIA from C. magus venom) inhibits a maximum of 65% of NE release and with much lower potency (0.5mM IC50). To understand the role of the conceptephide-sensitive components of NE release, slices were subjected to both peptides simultaneously: SNX-111 at a maximally (65%) effective concentration (30mM IC50) at which it exhibits only in high-potency (30%) inhibitory activity. Surprisingly, inhibition of NE release was not complete — being only about 85% complete. One explanation is that there are four conceptephide-sensitive components of NE release: total (50%), comp.1 (20%), comp.2 (20%), comp.3 (20%), comp.4 (10%).

SNX-230, 1mM 100% + + + +
SNX-230, 1mM 50% + + +
SNX-111, 100% 65% + +
SNX-230 + SNX-111 85% + + + +

These results indicate that the conceptephides SNX-111 and SNX-230 will be uniquely useful reagents in elucidating the roles of their calcium channel targets in neuronal function.

CALCIUM CHANNELS: PHYSIOLOGY II

410.1  

Release of caffeine-sensitive and IP3-sensitive intracellular Ca2+ stores are mediated by distinct proteins identified as ryoside receptor (RyR) and IP3 receptor (IP3R), respectively. RyRs in intracellular Ca2+ can induce release of Ca2+ from the caffeine-sensitive pool via the RyR, a process known as Ca2+-induced Ca2+ release. We have localized caffeine-sensitive and IP3-sensitive Ca2+ release channels in the brain using specific antibodies raised against purified brain RyR and IP3R, respectively. RyR was localized to pyramidal neurons at levels similar to low levels in the cortex. RyR was most enriched in the dentate gyrus and CA3/4 areas of the hippocampus, where IP3R was present at relatively low levels. In the cortex, RyR was localized to pyramidal neurons, some of which were not labeled by anti-IP3R antibodies. Antibodies to IP3R revealed the presence of RyR in axons and in dendritic spines receiving asymmetric synapses while IP3R was primarily identified in dendritic membranes forming symmetric synapses. These results suggest that the IP3- and caffeine-sensitive Ca2+ pools have roles in controlling intracellular Ca2+ levels that are in some cases distinct and that in other cases may interact in varying degrees depending on the neuron and the subcellular compartment within a given neuron. Supported by M01RR00933 to AHS, DA00074 to SLS, B-507-RR07062-26 to CA and Fitzger Fellowship to TMD. RPC is HHMI investigator.

410.2  
ACTION POTENTIAL-INDUCED ALTERATIONS IN CYTOSOLIC CALCIUM IN FURA-2-LOADED DISSOCIATED BULLFROG SYMPATHETIC NEURONS. T.J. Hepper and J.F. Fiekers, Dept. Anatomy & Neurobiology, College of Medicine, University of Vermont, Burlington, VT, 05405, U.S.A.

The kinetics of [Ca2+]i in response to direct electrical stimulation were examined in dissociated neurons from the 9th and 10th sympathetic ganglia of the bullfrog Rana catesbeiana. [Ca2+]i was determined in individual neurons using a ratiometric analysis with fura-2. Simultaneous recording of action potentials and passive electrical properties were recorded with electrophysiological techniques. Basal levels of [Ca2+]i ranged from 82 nM to 225 nM (n=19). Trains of action potentials (20 Hz) were evoked by depolarizing current injection. The rate of rise of [Ca2+]i was increased in a frequency-dependent manner. At each stimulus frequency, a maximal level of [Ca2+]i was obtained and maintained during the train. Basal levels were restored following cessation of stimulation. The addition of Co2+ (2 mM) to the extracellular solution abolished the stimulation-induced increase in [Ca2+]i. The [Ca2+]i response to afferent applications of ACh was decreased during train-induced elevations of [Ca2+]i. These results indicate that the origin of [Ca2+]i during action potential generation is extracellular and that these neurons are capable of buffering large changes in [Ca2+]i, during and following train stimulation. (Supported by NS 27319).

410.3  
SPONTANEOUS CALCIUM OSCILLATIONS IN CULTURED RAT CHROMAFFIN CELLS. A.R. Wakade, D.A. Przuvich, T.D. Wakade. Dept. of Pharmacology, Wayne State University, Detroit, MI 48202.

Rat chromaffin cells (RCC) of 8-day-old pups were used and were cultured to measure intracellular Ca2+ by Indo-1 dye method, to monitor membrane potential using patchclamp technique and to determine catecholamine release by labeling with [3H]NE. Almost all cultured RCC exhibited spontaneous oscillations (OSC) in intracellular free Ca2+ ([Ca2+]i). The OSC were random and varied in frequency, duration and magnitude. Neither calcium, caffeine, antagonist, ryoside, thapsagin nor tetrodotoxin or tetraethylammonium affected OSC. [Ca2+]i-free Krebs solution plus 0.5 mM EGTA completely blocked OSC and lowered [Ca2+]i in RCC. Spontaneous release of [3H]NE was reduced by more than 50% when Ca2+ was omitted from the external medium. Low temperature (2°C) caused maximum and sustained increase in [Ca2+]i. Whole cell current clamp recording showed that RCC had unstable resting membrane potentials (Em) and spontaneous action potentials (AP) which varied in frequency. Spontaneous AP were depolarized by TTX and blocked by TTX plus Ca2+ channel blockers. However, Em continued to fluctuate in the presence of these blockers. We have ruled out the possibility that Ca2+ OSC are due to voltage- or receptor-operated Ca2+ channels, or release of Ca2+ by intracellular mechanisms. Passive influx of Ca2+ could generate OSC and initiate exocytosis in RCC.

410.4  

We studied how [Ca2+]i relaxes after sudden changes in voltage-dependent Ca2+ entry, and uptake and release from a caffeine- and ryoside-sensitive store. High K+ (30-50 mM) depolarized Vm and caused a steady [Ca2+]i elevation, while caffeine (10 mM) produced a transient [Ca2+]i rise. Restoring K+ to 2 mM led to repolarization and a monotonic decline in [Ca2+]i. Caffeine removal produced a transient [Ca2+]i undershoot. Both relaxations are described by the sum of two decaying exponentials with the same t1/2 (3-5 s) and t1/2 (3-5 min) but with different amplitudes. This is consistent with a three-compartment scheme consisting of the external bath, the cytoplasm and an internal store, with pump and leak fluxes between compartments depending linearly on Ca2+ concentration, valid for small departures from the steady state. In this scheme, the r's reflect the dynamic properties of the system (transport rates, relative cytosolic and store volumes) while the amplitudes additionally depend on the initial conditions. To account for Ca2+ dynamics in the presence of caffeine, an internal Ca2+ dependent leak was introduced. The resulting flux functions exhibit periodic solutions resembling [Ca2+]i, oscillations in the presence of caffeine and high K+ (Friel and Tielin, 1992). Neurosci 8, 1-20: (1) tCa2+ " + frequency (f); (2) tCa2+ " + and amplitude, and (3) Ca2+ removal has phase-dependent effects on [Ca2+]i.

Therefore, a three compartment system with linear Ca2+ pumps and leaks and a single Ca2+-dependent leak in the internal store predicts [Ca2+]i oscillations like the observed ones; nonlinear pumps, etc. are not required. This provides a simple and testable model for caffeine-induced [Ca2+]i oscillations in sympathetic neurons, and may be useful in the study of caffeine- and ryoside-sensitive [Ca2+]i oscillations induced by agonists in non-neuronal cells.
410.5 DISTRIBUTION OF CALSKEQUIN, RYANOGEN AND IP3 RECEPTORS IN THE CHICK CEREBELLUM: A THREE-DIMENSIONAL IMMUNOCOLIZATION STUDY. M. E. Martone*, Y. M. Sappalagoni, T. Z. Dzczacka, J. A. Artzi†, L. J. Sztako†, and M. H. Hillman. San Diego Micromorphology Laboratory, UCSD, La Jolla, CA 92038; Pharmacology, Univ. of Nevada, Reno, NV 89557.

Many proteins involved in intracellular calcium regulation in skeletal or smooth muscle, e.g. the IP3 and ryano gen receptors, calskenein, Ca2+ ATPase, have been found to be abundantly expressed in the cerebellar perikarya (Vilin et al., J. Cell. Biol. 113: 739-745, 1991; Sztako et al., J. Cell Biol. 113: 739-745, 1991). While the muscle sarcolemmal reticulum is planar and ordered relative to the sarcomere, the neuron endomembrane system anastomizes in three dimensions (3D) presenting formidable obstacles to understanding the organization of calcium regulatory components. In the present study, the distribution of immunolabeled IP3 receptor (IP3R) and ryano gen receptor (RyR) was examined in chick cerebellum. Labeling for these proteins was determined in E18 to E17 chick cerebellum using laser-scanning confocal microscopy. Although labeling for all three proteins was extensively distributed within Purkinje cells, a unique pattern was found for each. Labeling for the IP3R was fairly evenly distributed throughout the cell body, dendrites and dendritic spines while labeling for RR and particularly CS was more discontinuous and not found in spines. The discontinuities in CS labeling appeared most prominent in older chicks. Preliminary analyses of double-labeled preparations suggest that the distribution of RR and CS labeling are not identical. In order to determine the structural basis for this difference, we have adopted a cryoimmunocytochemistry imaging method for 3D studies using thin sections and high voltage electron microscopy. Labeling for IP3R with 5nm gold was achieved through 0.25 um sections, with good structure preservation at normal cryo-conditions and elevated temperatures. Through the use of single and double-labeling, we expect to resolve how the spatial distribution of these proteins relates to the endomembrane system components thus providing important information to our understanding of the mechanisms by which these cells modulate intracellular calcium.

410.7 AN ALLOSTERIC MODEL FOR INACTIVATION OF VOLTAGE-DEPENDENT CHANNELS. Stephen W. Jones‡, Dept. Physiol. & Biophys., Case Western Reserve Univ., Cleveland, OH 44106.

Activation kinetics of L-type calcium channels can be described based on the Monod-Wyman-Changeux model, with movement of a voltage sensor corresponding to ligand binding, and channel opening corresponding to activation of the protein; the channel opens more readily with more voltage sensors moved (Marks & Jones, J. Gen. Physiol. 99, 367-390, 1992). A model where inactivation is also allosterically coupled to voltage sensor movement can explain (1) voltage-dependent macroscopic inactivation, (2) voltage-independent microscopic inactivation, (3) activation showing significant depolarized voltages, but rapid recovery from inactivation at negative voltages, and (4) Vv0 for inactivation more negative than Vv0 for activation. In its simplest form, where inactivation rates depend on how many voltage sensors have moved but not on whether the channel is open or closed (or on voltage), the model has 9 free parameters.

410.8 DETERMINATION OF THE 3-D CHANGES IN INTRACELLULAR Ca2+ ACTIVITY IN DISRUPTED RAT HICCUPPAL NEURONES USING RATIMOMIC FOCAL LASER SCANNING MICROSOPY. S. Jams, R. Perro, H. V. Wheel & J. E. Chaf, Dept. of Physiology and Pharmacology, Univ. of Southampton, SO1 3TU, UK.

The increase in intracellular calcium ion activity (Ca2+) within neurones is a powerful and potentially dangerous event for the regulation of neuronal activity and can lead to cell death. However, conventional investigative techniques have lacked the resolution to determine the true spatial and temporal extent of these changes. Confocal Laser Scanning Microscopy (CLSM) can resolve cellular morphology to a sub-micron level in three dimensions (3-D), revealing individual synaptic spines. This capability can be harnessed to the determination of changes in intracellular Ca2+ ion activity (within discrete volumes; focal plane) with the use of Ca2+ sensitive fluorescent dyes. We have used the acetyoxymethyl esters (AM) of the calcium-sensitive fluorescent dyes fluo-3 (10μM) and indo-1 (0.5μM), to load (60min,18°C) viable, dissociated rat hippocampal neurones. Indo-1 requires UV (360nm) excitation optics (argon ion laser), but can be used for ratiometric measurements. The Ca2+ responses of neurones to elevated K+ (20mM), caffeine (20mM) and glutamate (10μM) were tested (excitation lens Nikon 60 Plan Apo, NA 1.4). The experimental design was to record Z-series image data under control conditions, in the presence of the stimulus, after wash, in the presence of ionomycin, and finally with ionomycin and extracellular Ca2+ removed. The two last give a (maximal (saturated) and minimal Ca2+ loading of the dye. Caffeine gave the most consistent data, with apparently widespread elevations in Ca2+ (n=20/20 viable cells). Both glutamate and high K+ also produced increases in Ca2+ but less consistently. We are presently refining our techniques to unequivocally determine the subcellular localisation and extent of the changes in Ca2+ due to different stimuli.

Supported by SERC, MRC and University of Southampton.

410.10 ISOLATION OF NOVEL NEURONAL CALCIUM CHANNEL BETA SUBUNITS. E. Tunza and M. D. Uhler. Graduate Program in Neuroscience, Mental Health Research Institute and Department of Biochemistry, Mental Health Research Institute, Ann Arbor, MI 48105.

The dihydroxyproline-sensitive calcium channels in rabbit skeletal muscle consist of α1, α2, β, and γ subunits. The α1 subunits of these skeletal muscle subunits have been cloned. The β subunit has been further characterized and shown to have two genes that encode for homologous protein subunits. β1 is expressed in skeletal muscle and brain. In contrast, β2 is expressed in brain, heart and lung. In order to isolate splice variants of the β2 subunit, we screened a mouse brain cDNA library with a 900 bp DNA fragment corresponding to the first 900 nucleotides of the rabbit skeletal muscle β subunit coding region. Preliminary characterization of the isolated cDNA indicates that multiple splice variants of the β2 exist in brain. Sequencing of one clone demonstrated the existence of a previously undescribed splice variant. We have isolated a unique, new variant of the α1 subunit terminal sequence of this splice variant was found to diverge from the published α1 subunit sequence at amino acid 17. Initial characterization of the other clones suggests that additional splice variants of the α1 subunit exist in brain. In the future, crossexpression of these novel α1 subunit splice variants with mouse neuronal α1 subunits, currently being cloned in our laboratory, will be performed.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
410.11 ISOLATION AND CHARACTERIZATION OF THE P-TYPE CALCIUM CHANNEL FROM MAMMALIAN MUSCLE. B. Cherkesy, M. Sugimori and R. Linz. Dept. Physiol. & Biophys., NYU Med. Center, New York City, NY. P-type calcium channels, which are specifically blocked by the polyamine channel blocker "syntonic FTX", have been shown, both functionally (Linz et al, PNAS, 1989) and immunohistochemically (Linz et al, J. Histochem. Cytochem., 1991), to be present in different regions of the CNS. However, little is known about their distribution outside of the brain. The polyamine, l-arginyl-NO-,N′,N′-diisopropyl-1,4-butanediimine, which we have previously been employed to isolated synaptosomes of Sepharose 6B CL via a 1,4-Butanediol Dipycyl ether linkage. This affinity gel allowed the extraction of a protein from rat synaptosomal skeletal muscle homogenate (10% w/v). The functional activity of the resulting protein solution was assessed using the "up-down" bilayer technique. The electrical activity was measured in solutions containing: 80 mM BaCl2, 10 mM HEPES, pH 7.4 in the pipette; 0.25 M sucrose, 10 mM HEPES, pH 7.4 in the bath. Single channel activity was recorded under these conditions was characterized by a slope conductance of 55 fA. The current-voltage relationship was linear above -100 mV. The channel was rarely open at potentials more negative than -70 mV and opened with increasing frequency as the potential was made more positive. Single channels were blocked by the addition of Ca or Ca concentrations below 100 µM and by both a polyamine blocker synthetic FTX and by natural FTX. Ensemble averages of 60 ms steps from -30 to +30 mV showed that the channel activity did not inactivate during the 200 msec pulse duration. Analysis of the protein solution isolated using the polyamine affinity gel was performed using SDS-PAGE in polyacrylamide gels (only a single band (a doublet structure) at a molecular weight of 80-150 kDa was found when gels were silver stained. The results have shown that the protein isolated from mammalian skeletal muscle using an FTX affinity gel is a P-channel of similar structure and behavior to that found in CNS. Supported by NIH grants NS13742 and AG09480.

410.13 P-CHANNEL IS RESPONSIBLE FOR HIGH-THRESHOLD DENDRITIC ACTION POTENTIAL IN INFERIOR OLIVE NEURONS, AN FTX STUDY. A. Manfredi, B. Cherkesy, M. Sugimori, and R. Linz. Dept. Physiolology and Biophys., NYU Medical Center, 550 First Avenue, NY, NY 10016. Inferior olive cells have been shown to exhibit high-threshold and low-threshold calcium spikes (Linz et al, J. Physiol., 315:549,1981). The low-threshold spike is generated by a conductance consistent with the presence of T-type voltage-dependent calcium channels. The type of channel responsible for the high-threshold spike has not been determined. A histochemical study of the distribution of P-channels in the CNS using P-channel specific antibodies indicated that the inferior olivary neurons express P-channel (Manfredi et al, J. Neurochem., 68:7076, 1998). Since a polyamine purified from Agelopoge Aperta (FTX) has been shown to block P-channels (Linz et al, PNAS 86:1689, 1989) the effect of HPLC-purified FTX as well as whole venom of Agelopoge Aperta was tested in intracellularly recorded inferior oliver neurons in guinea pig brain stem slices. Both of these substances produced a clear block of the high-threshold calcium spike without affecting either the sodium action potential, the low-threshold calcium spike or calcium-dependent potassium conductance that generates the afterhyperpolarization. By contrast, the large high-threshold calcium dependent action potential and the afterhyperpolarization that follow were blocked. In addition, when the cell was depolarized, repetitive high-frequency sodium dependent action potentials could be obtained. The results indicate at least two calcium conductances are found in the inferior olive, a P-type conductance responsible for the high-threshold spike and the P-type channel responsible for most of the high-threshold spike. (Supported by NIH grants AG09480 and NS13742)

410.12 CHARACTERIZATION OF P-TYPE CALCIUM CHANNELS IN CEREBELLAR PURKINJE CELLS. M.M. Ussizig*, M. Sugimori, R. Cherkesy & R. Linz. Dept. of Physiology and Biophysics, NYU Medical Center, 550 First Avenue, New York, NY. There is good agreement between the pharmacological profiles of P-type Ca channels in cerebellar Purkinje cells, Ca channels expressed in Xenopus oocytes from brain homogenates, and the P-type Ca channel (see Tieson et al, TIPS 12, 349). However, it has been difficult to compare the biophysical properties of these channels, because the currents have not been recorded with the same Ba concentrations. Therefore, we have recorded P-type Ca channel currents carried by Ba ions ranging in concentration from 1mM to 100mM, and also by 2.4mM Ca. Cell-attached patch recordings were made at 22°C from the somas of adult Purkinje cells in thin cerebellar slices of the guinea-pig (Edwards et al, Piongues Archiv 414:600). The threshold of activation was carried linearly with the log of [Ba]; it was shifted towards positive potentials as [Ba] was increased, presumably due to screening of the surface potential by Ba ions. For instance, it was -40mV with 2.2mM Ba, -41mV with 5mM Ba and -23mV with 40mM Ba. Currents carried by 2.4mM Ca activated at around -41mV. Moreover, we found little difference in single-channel conductances measured with 10-100mM Ba; channel openings were to three conductance levels of 10pS, 14-16pS and 19-21pS (slope conductances). As [Ba] was reduced below 10mM, the unitary currents became progressively smaller and multiple levels could not be resolved. Single-channel currents carried by 2.4mM Ca were barely resolved. Supported by NINDS grant NS 13742 and at NESC/NOF Fellowship (MMU)

410.14 IMMUNOLOGIC AND KINETIC CHARACTERIZATION OF A Na+/Ca2+ EXCHANGER IN PRIMARY NEURONAL CELL CULTURES. G. Hoer, R. Pat, M. Herrbert, J. Wenzel. Dept. Pharmacology and Toxicology, and Center for Biomedical Research, University of Kansas, Lawrence, KS 66045. The plasma membrane Na+/Ca2+ exchanger is believed to play a role in regulation of Ca fluxes in neurons. We recently reported the development of Ab's against a 36 kDa synaptic membrane protein which immunoprecipitated an exchanger activity from purified dissociated membranes (J. Neurochem. 58:147, 1992). We have now used these Ab's to label primary neurons in culture with avidin-biotin conjugates to peroxidase. The antibodies produced significant labeling of axons, dendrites and cell bodies in neuronal cultures prepared from 18-day embryonic rat brain. Given the substantial amount of Ab labeling of the cells, it was of interest to see whether these cells exhibited Na+/Ca2+ exchanger activity with properties similar to those reported for adult brain synaptic membrane preparations. Points of contact between neurons stained quite heavily. Sodium-dependent Ca2+ transport was measured in washed homogenates of primary cortical neurons maintained for 3-6 days in serum-free, defined culture medium. Kinetic determinants of transport activity revealed that Ca2+ uptake was linear for at least 30 seconds at 23°C. Uptake was measured across Ca2+ concentrations using 50 sec incubations and the kinetic constants were determined to be: Km = 39 µM and the Vmax = 0.55 nmol/mg protein/sec. These values are quite close to those reported for other, somatic brain preparations. (Supported by grant AA04732 and MDM Sci. Educ. Partnership.)

ACETYLCHELORINE: CNS I

411.1 AFFERENTS TO THE MESOPONTINE CHOLINERGIC NUCLEI FROM THE PERIPHERAL BULBARY IN THE RAT. T.L. Steininger and B.H. Walker, Comm. on Neurobiology, and Dept. Pharm. and Phys. Sci., The University of Chicago, Chicago, IL 60637. A large body of evidence suggests that the mesopontine cholinergic groups, the pedunculopontine tegmental (PPT) and laterodorsal tegmental (LDT) nuclei, participate in mechanisms of behavioral state control; specifically in arousal and the rapid eye movement sleep. Previous retrograde tracing studies have identified putative afferents to the PPT from numerous regions, including the brainstem, olfactory bulb, striatum, hypothalamic area, dorsal raphe nucleus, and the periaqueductal gray (PAG). Furthermore, the ventrolateral region (Steininger et al., J. Comp. Neurol., in press). In the present study, we have utilized anterograde tracing with PHA-L coupled with choline acetyltransferase immunohistochemistry to examine the PAG innervation of the mesopontine tegmental nuclei. At the light microscopic level, a dense innervation of PHA-L labeled fibers was observed in the region of the PPT and LDT. At higher magnification, the fiber terminals were observed between anterogradely-labeled boutons and cholinergic neurons. The functional implications of these connections are unclear, but may be involved in mediating the physiological and behavioral correlates of arousal that are features of the "defense reaction" elicited experimentally by ventralpial PAG stimulation. (Supported by NS 17661 and MH 09919)

411.2 MEDIAL PREFERENCES CORTEX PROJECTS DIRECTLY TO CHOLINERGIC NEURONS OF THE MESO-PONTINE TEGMENTUM. R.S. Reves*, S.R. Grant. Dept. of Biological Sciences, Psychology and Program in Neuroscience, University of Delaware, Newark, DE 19716. Stimulation of the medial prefrontal cortex (MPFC) reliably activates neurons in meso-pontine nuclei including the laterodorsal tegmental nucleus (LDT) and rostral locus coeruleus (rLc). Although anatomical studies report direct projections from the MPFC to the LDT it is not clear if these cortical afferents directly and/or selectively innervate cholinergic neurons in the LDT. In order to resolve this question we used Phaeusolus leucogatullin (Phal.) injected into the rat MPFC to labelafferent fibers, and NADPH-diaphorase histochemistry to identify cholinergic neurons. Later, both cholinergic and non-cholinergic neurons in the LDT were contacted by numerous branched and varicose affenter fibers. The fibers were densely at rostral levels and formed a continuous plexus that extended from the LDT to the adjacent dorsal raphe (DR), but avoided the intervening dorsal tegmental nucleus of Gudden. Some fibers swept laterally into the dorso medial portion of the pedunculopontine tegmental nucleus, and extended ventrally into the reticular formation. Laterally, fibers generally spared Barrington nucleus, but Phal. positive fibers occasionally entered the rLC. Fibers in the LC appeared thicker than fibers in other regions. The relative number of Phal. labeled fibers in the LDT and LC was related to the domino- ventral positioning of the injection. This study is consistent with our stimulation and pharmacological studies suggesting a monosynaptic excitatory amino acid projection from the MPFC to the LDT. Studies combining NADPH- diaphorase immunohistochemistry are in progress to verify the cortical transmitter. Supported by NIMH, the State of Delaware, and ICI Pharma.
411.3

ACETYLCHOLINE: CNS I

411.4


The descending cholinergic projection from the laterodorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei to the pontomesencephalic reticular formation is thought to be implicated in the regulation of the rapid-eye-movement (REM) sleep phase. The fluorescent triple-labeling technique combined choline acetyltransferase (Chat) labeling by fluorescent (AMCA) immunohistochemistry with the use of two different fluorescent retrograde tracers, fluoroscan- and cholodin-conjugated latex beads. The retrograde tracers were injected into the entire side of the pontomesencephalic reticular formation and adjacent structures in 13 rats. In each case, triple-labeled neurons were found in the LDT and PPT nuclei, indicating Chat immunopositive neurons with bifurcating axons. Overall, there was a contralateral projection in about 22% of all ipsilaterally projecting neurons in the pons and 15% in the medulla for the PPT. Furthermore, for contralaterally projecting PPT neurons, there were ipsilateral branches in 38% in the pons and 22% in the medulla. LDT percentages were less in both cases. Quantitative volumetric measurements indicated that all injections of retrograde label occupied less than 3% of the reticular nuclei; despite this small volume of injection, bilaterally projecting cholinergic, an oxytocinergic, and a vasopressinergic neuron population was found. In summary, our data support the idea that a substantial number of cholinergic neurons in the pontomesencephalic reticular formation project bilaterally, and that cholinergic neurons are involved in the regulation of the rapid-eye-movement sleep phase.

411.5


Intracellular recordings were made from laterodorsal tegmental nucleus neurons in the in vitro brain slice preparation. Recordings were obtained from 95 cells which had mean input resistances of 160 (+/- 45) MOhm and action potential overshoots of at least 10mV. Virtually all cells, regardless of their cholinergic or non-cholinergic nature, responded to the application of carbachol (1-10μM) with a membrane hyperpolarization and increased decrease in input resistance. Under voltage clamp conditions this response was shown to be due to an outward current that was strongly inward rectifying and had a reversal potential near the equilibrium potential for potassium. The carbachol response was partially blocked by cesium (2nM) and fully blocked by barium (100μM) providing additional evidence for the activation of an inward rectifying potassium conductance by carbachol. The carbachol effect was not blocked by low concentrations of the M1 antagonist pirenzepine, indicating that the response was due to the activation of non-M1 acetylcholine receptors. Supported by the Dept. of Veteran's Affairs and N.I.M.H. grant MH39863.

411.6

NORADRENALINE HYPERPOLARIZES CHOLINERGIC NEURONS IN RAT LATERODORSAL TEGMENTUM IN VITRO. J.A. Williams* and P.B. Reiner Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, University of British Columbia, Vancouver, B.C. Canada, V6H 1Z1.

Inhibition of brainstem cholinergic neurons by noradrenergic neurons of the locus ceruleus has long been suggested as one mechanism of behavioral state control. While there is anatomical evidence for a noradrenergic innervation of cholinergic neurons in the rat laterodorsal tegmentum (LDT), the direct inhibitory influence of noradrenaline on cholinergic neurons has never been demonstrated. The purpose of the present study was to characterize the effect of noradrenaline upon cholinergic neurons in the rat LDT. Using whole-cell patch clamp recordings in slices, 26 cells were studied during bath application of 50 μM noradrenaline (NA). Cholinergic neurons were positively identified by intracellular labelling with biocytin and subsequent NADPH-diaphorase staining. 93% (13/14) of identified cholinergic neurons hyperpolarized with an increase in conductance in response to noradrenaline. In contrast, non-cholinergic neurons exhibited mixed responses to NA (25% (3/12) hyperpolarized, 33% (4/12) depolarized, 42% (5/12) no response). Bath application of 1μM isoproterenol blocked the hyperpolarizing effect of NA on cholinergic cells. To test whether the response to NA was a direct effect, some slices were bathed in low Ca2+, high Mg2+ ACSF solution. The responses of cholinergic cells to NA in this condition were identical to those in normal ACSF. These results demonstrate for the first time the direct hyperpolarization of cholinergic neurons by NA, and that this is an oxy-mediated effect.

supported by J.O.D.E. and B.C.H.C.R.F.

411.7

EFFECTS OF SEROTONIN UPON BASAL FOREBRAIN NEURONS IN VITRO. Natalia A. Gorelova* and Peter B. Reiner, Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, BC V6T 2Z3 Canada.

Neurons of the basal forebrain project to the hippocampus and cerebral cortex and are thought to be intimately involved in the control of cortical arousal. Anatomical studies have demonstrated the existence of serotonergic afferents to the basal forebrain. Moreover, there is considerable evidence that serotonergic inputs to the medial septum are involved in generation of some types of hippocampal theta rhythm. In order to understand the role of serotonergic afferents in control of cortical arousal, we studied the responses of basal forebrain neurons (medial septum, diagonal band) to serotonin using whole-cell patch clamp recordings in an in vitro slice preparation. Basal forebrain neurons exhibited heterogeneous physiological properties, with a mean resting membrane potential of -52 ± 4.8 mV and input resistance of 350 ± 152 MΩ. In accord with the variable electrophysiological properties of basal forebrain neurons, differential, of twelve cells studied, 6 cells depolarized, 2 cells hyperpolarized and 4 cells showed no response. Current efforts are directed towards correlating transmitter status with responses to serotonin.

[Supported by the Medical Research Council of Canada]

411.8

NUCLEUS BASALIS STIMULATION ELICITS NEOCORTICAL ACTIVATION AND FACILITATES THALAMOCORTICAL SYNAPTIC TRANSMISSION: INTRACELLULAR AND EXTRACELLULAR RECORDING IN RAT AUDITORY CORTEX. Paul Medrano* and John H. Ashe, Departments of Neuroscience and Psychology, University of California, Riverside. CA 92521.

Neocortical activation (EEG) during basal nucleus stimulation) reflects a state of behavior and cortical information processing. Through largely undetermined cellular mechanisms, the neurotransmitter acetylcholine (ACh) is thought to be involved in neocortical activation. Since nucleus basalis (NB) neurons are a primary source of cortical ACh, these cells may mediate EEG activation. We have examined this hypothesis, and the implications of cortical activation for thalamocortical transmission, by stimulating the NB and the auditory thalamus (medial geniculate, MG) during intracellular and extracellular recordings in the middle to deep layers of auditory cortex in urethane-anesthetized rats.

Electrical or chemical stimulation of the caudal NB desynchronized the local EEG recorded in auditory cortex; this action was blocked by cortical application of the muscarinic receptor antagonist atropine. Simultaneously with its effect on the EEG, NB stimulation elicited depolarization (-10 mV) of cortical neurons and subthreshold membrane potential fluctuations from large amplitude, slow (1-5 Hz) oscillations to low amplitude, fast (25-40 Hz) oscillations. MG stimulation elicited a discharge in auditory cortex and afferent volley (peak latency ca. 2 ms) followed by an afterhyperpolarization (ca. 3 ms onset, 7 ms peak) by a negative-going field potential, or intracellularly, an excitatory postsynaptic potential (EPSP). NB stimulation did not alter the thalamocortical afferent volley, but facilitated the slope and amplitude of the negative-going field potential via a muscarinic action. NB stimulation also enhanced MG-evoked EPSPs, producing a hyperpolarization that was followed by a slow afterhyperpolarization.

These data suggest that one function of ACh-mediated neocortical activation is to facilitate thalamocortical transmission.

Supported by the NSF.
411.9 Ca²⁺-CALMODULIN KINASE MEDIATES MUSCARINIC BLOCKADE OF GluCa and ENHANCEMENT of Ca²⁺ CHANGES IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS. W. Muller*, J.J. Petrozzi,* L. Griffith,* W. Danhof* and J.A. Corporan* Dept. of Neurosciences, Roche Center, Hoffmann-LaRoche, Inc., Roche Research Center, Nutley NJ 07110

An important intrinsic control of excitability in pyramidal neurons of the hippocampus is represented by adaptation of discharge during constant-current flow afterhyperpolarization following repetitive discharge. This negative feedback is mediated by Ca²⁺ dependent K-conductances GluCa and is subject to down-modulation by extracellular muscarinic agonists. Muscarinic suppression is of particular interest for its important role in synaptic plasticity and learning. This input uncouples intradendritic Ca²⁺ from synaptic activation and thereby potentiates intradendritic Ca accumulations, a factor that possibly underlies facilitation of synaptic plasticity during muscarinic activation.

Using new peptide inhibitors of Ca²⁺-calmodulin dependent kinase (CaM kinase) and PKC we found that muscarinic modification of GluCa and Ca²⁺-changes relies on CaM kinase activation, but the converging effects of serotonic and glutamatergic agonists are mediated either through other kinases or by different intrinsic mechanisms. We summarize that these large part separate pathways include also different branches that are relevant for distinct functions of these neurotransmitters.

W. Muller was supported in part by a Helmholtz-Stipend from the BMFT.

411.11 CHOLINERGIC SLOW SYNAPTIC ACTIONS ON RAT NEOCORTEXAL NEURONS IN VITRO. J.H. Asher*, C.L. Cox, R. Mehler and E. Jaber. Deps. of Neuroscience and Psychology, Univ. of California, Riverside, CA 92521.

Acetylcholine (ACh), exogenously applied, can produce lasting modifications of functional characteristics of cortical neurons. However, the actions of synaptically released ACh upon neuronal excitability and synaptic transmission in neocortex are not clearly understood. These experiments investigated the action of cholinergic agonists and endogenous ACh upon auditory cortical neurons.

Tetanic stimulation of deep cortical gray matter (10-100 mA; 8-40 pulses, 20-80 Hz) elicited a voltage-dependent long-lasting slow membrane depolarization (tonus of seconds) to many microelectrodes. This slow depolarization persisted in the presence of amino acid antagonists (DNQX, APV, picrotoxin). Tetanic stimulation also produced voltage dependent 1) decreased spike frequency accommodation, 2) decreased slow afterhyperpolarization, and 3) facilitated spike afterdepolarization. The effect of the tetanic stimulation were mimicked by exogenously applied cholinergic agonists, ACh and mecameline (Mecamylamine, mecamylamine), and antagonized by the muscarinic antagonists atropine or pirenzepine. These data suggest that synaptically released ACh produces multiple voltage-dependent actions via muscarinic receptors.

A subpopulation of neurons has been identified as "thyroid-bursting" neurons due to their low frequency rhythmic (5 Hz) bursting discharge to a depolarizing current pulse. These cells have an intermediate firing pattern identified by layer 5 pyramidal neurons following bicuculline injection. In these cells, tetanic stimulation reduced the number of bursts and increased the number of higher frequency (10-15 Hz) single spike discharge in response to a depolarizing current pulse, independently of membrane depolarization. This effect was enhanced by exercise, blocked by atropine and mimicked by muscarinic agonists, Mecamylamine and Carbachol.

These data suggest that synaptically released ACh, acting via muscarinic receptors, can modulate the excitability of neocortical neurons. Modulation of the excitability of these neurons may influence the neurons response to subsequent afferent and efferent information and may serve as a potential mechanism of synaptic plasticity. Supported by NSF BNS 9008618.


Neocortical cells recorded from cat association areas 5 & 7 display an oscillation around 0.5 Hz, consisting of depolarizing and hyperpolarizing sequences, time-locked to a similar EEG oscillation (Steriade et al., this meeting). Here we report the modulation of this novel type of slow neuronal oscillation by shifting the EEG synchronization (high-amplitude and slow waves) to activated patterns. We used intracellular recordings of cortical cells under urethane anesthesia to examine the effect of the EEG state by a brief stimulation (pulse-trains at 30 Hz lasting for 1 s) of pedunculopontine tegmental (PPT) and locus coeruleus (LC) nuclei. In most cells, PPT stimulation produced a depolarization (1-5 mV) associated with tonically increased firing and lasting for 10-20 s. The slow oscillation was blocked for 5-10 s and resumed thereafter. This cellular effect was voltage-dependent, as it could not be obtained at a membrane potential more negative than -45 mV. In some neurons, in which the oscillation mainly consisted of rhythmic hyperpolarizations superimposed by depolarizing waves, PPT stimulation interrupted the oscillation by selectively suppressing the hyperpolarizing episodes. The time course of the PPT-induced blockage of slow oscillation was similar to that of EEG activation. The PPT effect was suppressed by systemic administration of a muscarinic blocker, scopolamine. LC stimulation produced qualitatively similar effects on cells and EEG activity, but less pronounced and durable. After clonidine administration, the effects of LC stimulation were no longer observed. The similarity between the cholinergic and noradrenergic effects suggests that the major mechanism underlying the blockage of the slow cortical oscillation, and especially of the rhythmic hyperpolarization, is the reduction or suppression of potassium conductances. Supported by NRC of Canada (grant M3869).

411.13 SERIAL SECTION ANALYSIS OF THE ACETYLCHOLINE (ACH) INNERVATION IN ADULT RAT PARIETAL CORTEX. D. Unbom*, K.C. Watkins*, D.H. Hart* and S. Czerny CRSN (Départements de pathologie et de physiologie), Université de Montréal, Montréal, Qc, CANADA; Instituto Biologia Cellulaire CNR, Roma (ITALY), and Department of Psychiatry, University of Minnesota, Minneapolis (MN).

We have now completed the electron microscopic examination in serial thin sections of 795 portions of adult rat parietal cortex (ParI), immunostained with monoclonal antibodies against purified rat brain choline acetyltransferase (Soc. Neurosci. Abstr. 16:300, 1990). This material from 4 rats was initially fixed by perfusion of a paraformaldehyde (2.5%) - glutaraldehyde (0.1%) mixture, and processed with the ABC method. 140 varicocities of layers I, II, III, IV and V, 517 of layer IV, 199 of layer V, 216 of layer VI were photographed from end to end and examined at a final magnification of 8000 (average of 11 pictures for varicosity). The maximal transverse diameter of these varicosities was similar in every layer (0.49 ± 0.15 μm s.d.). Both large and small varicosities could be observed on the same fiber. The average proportion of the varicosities exhibiting a synaptic membrane differentiation (9.3% in layer I; 13.8% in II-III; 11.6% in IV-V; 26.2% in VI), which reaches a maximum of 14.5%. These 118 synaptic junctions were almost invariably symmetrical (98.2%). A majority were found on dendritic branches (80.7%), some on spines (22.8%) and none on cell bodies. The varicosities located on the same fibers. There were only 4 varicosities with dual junctions and the 2 asymmetrical junctions were on spines in layers I and II-III. These data indicate that the ACh nerve terminals form an punctate, with a junctional, with a slightly higher proportion of synaptic varicosities in layer V. Identification of receptive elements will be needed to determine the functional targets of such an innervation. [Supported by the FCAR and grants MT-3544 (MRC) and NS 12311].


The cholinergic innervation of the human amygdaloid complex was studied using choline acetyltransferase (ChAT) immunohistochemistry. CHAT-positive fibers and varicosities were observed throughout the amygdaloid complex. In parts of the amygdala the density of this cholinergic innervation was higher than in any part of the cerebral cortex. The highest level of ChAT-positive varicosities was seen in the basolateral nucleus, the lateral nucleus of the amygdaloid complex. The basomedial, accessory basal and cortical nuclei, the amygdaloparietal area, and the amygdalopeduncular transition areas and anterior amygdaloid areas showed a lower density of CHAT-positive varicosities and fibers. The lateral nucleus displayed the highest level of innervation and there were only rare CHAT-positive fibers in the medial nucleus. The two lateral nuclei together displayed a low level of innervation in comparison to the other amygdaloid nuclei. The amygdaloid complex is approximately equivalent to that of entorhinal cortex, which receives one of the heaviest of all extrinsic innervations in the cerebral cortex. The pattern of differential staining was maintained throughout the anteroposterior extent of the amygdala. The cholinergic innervation of CHAT-positive fibers and varicosities within the various nuclei and their subdivisions was by and large homogenous, with a given level except for some patches in the central nucleus. The distribution of CHAT-positive fibers was determined using the post-mortem sections as studied by CHAT immunohistochemistry and was comparable to that observed with ACHE histochemistry. These results confirm that, as part of the limbic system, the human amygdaloid complex receives a dense and differentially distributed cholinergic innervation.
411.15 ULTRASTRUCTURAL EVIDENCE FOR DISTINCT CHOLINERGIC AND VIP-ERGIC MODULATION OF INTRACRANIAL BLOOD VESSELS. A. Chebat*, D. Umirović*, B-K. Hartman* and E. Hamel*. Montreal Neurological Institute, McGill Univ., Univ. de Montréal, Montréal, Québec, Canada and Dept. of Psychiatry, Univ. of Minnesota, MN, USA.

Acetylcholine (ACH) and vasoactive intestinal polypeptide (VIP) exert potent vasoactive effects within the cerebral cortex. The relationships of ACh and VIP terminal profiles with cortical microvessels were evaluated at the ultrastructural level after immunocytochemical labeling for choline acetyltransferase (ChAT; MCAT-1C; Cozzari et al., Soc. Neurosci. Abstr. 16: 200:1990) or VIP (Pabst 4P8) with 4% paragamneldehyde (PF) and 0.025% glutaraldehyde followed by PF alone. Vascular terminals (section thicknesses 60 µm) were immunostained for ChAT or VIP by the ABC method with DAB-nickel as the chromogen. An equal number (> 80) of ChAT and VIP terminals located close (0.3 µm) to blood vessels were photographed and analyzed in single thin sections (Bioquant II). These terminals were respectively located at an average distance of 0.62 ± 0.05 and 0.75 ± 0.07 µm from the vessel wall (capillaries or arterioles). Of these, 26% (ChAT) and 20% (VIP) were separated from the basal lamina by a thin glial leaflet only, irrespective of the distance, VIP (0.56 ± 0.05 µm) were significantly larger than ChAT terminal (0.34 ± 0.03 µm; p < 0.001). Membrane specializations were never observed at the neuro-vascular interface even in serially-sectioned ChAT (n = 20) and VIP (n = 5) terminals. Together with our previous data showing that 59% of cortical neurons stain for VIP, 28% for ChAT and 13% only colocalize both transmitters, the present results strongly suggest that intracortical vascular functions are primarily influenced by distinct ACh and VIP nerves. Supported by the MRC of Canada.


NBOX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline), a selective antagonist of the AMPA subtype of glutamate receptor, has antispastic effects in monoamino-depleted rats and MPTP-treated primates (Ann. Neurol. 1991; 30:717-723). In the present study, NBOX was administered to normal rats and primates to determine if it produced any apparent side effects. In rats, various doses of NBOX (0, 5, 10, 20 mg/kg; i.p.) were evaluated (n=9/dose). Each rat was tested measuring horizontal activity and stereotypies. There were no apparent side effects in rats treated with 0, 5, 10 mg/kg NBOX. However, there was a slight but significant (p<0.05) narcotic effect observed in rats treated with 20 mg/kg NBOX. In the primate testing, 3 normal older (approximately 12-16 years old) female rhesus monkeys were administered doses of 0 and 1 mg/kg (i.m.) NBOX on alternate days. Each primate was subjected to 1 week of testing. Data were gathered by videotaping and computerized cage activity monitoring in which the number of times an animal crossed an infrared beam was recorded. These data were analyzed using a Clinical Rating Scale consisting of 4 measures of parkinsonian features (posture, gait, bradykinesia and balance), 3 measures of drug-related side effects (dyskinesias, vomiting and psychomotor disturbance) and 1 measure of overall level of activity. There were no apparent NBOX related side effects seen in any of the primates. Supported by a grant from the Motor Neuron Foundation.


Excitatory amino acid receptors can be classified by pharmacological and electrophysiological selectivity for the ligands NMDA, AMPA, kainic acid and t-ACPD. The present report characterizes the effect of antagonism of the AMPA subtype, NBOX, on local cerebral glucose utilization (LCGU). NBOX has been previously shown to have neuroprotective effects. NBOX was administered at doses of 3 to 90 mg/kg i.p. 30 minutes prior to the administration of 60 uCi [14C]-2-deoxyglucose i.v. in conscious rats. NBOX (3 to 30 mg/kg) selectively increased LCGU in the lateral heperula, raphe, superior and inferior colliculus and vestibular nucleus. NBOX at 60 mg/kg decreased LCGU in 47 of the 62 brain regions examined. NBOX at 90 mg/kg decreased LCGU in 60 of the 62 brain areas examined.

NBOX did not increase LCGU in limbic brain areas at any of the doses examined. In fact, NBOX at ≥60 mg/kg decreased LCGU in limbic areas. These data suggest that antagonism of the AMPA receptor subtypes with NBOX results in changes in LCGU different from those previously reported for non-competitive antagonists (i.e. PCP, MK 801), further suggesting that NBOX may not be associated with PCP-like effects.

412.3 KAINATE-INDUCED EPILEPSY LEADS TO AN INCREASE IN GLUTAMATE-STIMULATED PHOSPHODIESTERASE METABOLISM IN THE HIPPOCAMPUSS. M. Maya*, M. Emin-Matal, N. B. DallaMora, and R. Recasens*. INSERM U239, Hopital St-Charles, INSERM U248, CNRS UPR 8402, 34000 Montpellier, FRANCE.

Intrahippocampal injections of kainate (KA) ultimately induce status epilepticus associated with neuronal loss in local and distant structures. In the hippocampus, a mossy fiber sprouting occurs in consequence of this seizure-related damage. Since glutamate metabotropic receptors (mGLU-R) may be involved in synaptic plasticity, we examined the agonist-induced inositol phosphates (IP) formation in hippocampal slices of rats subjected to an intrahippocampal KA injection (2.5 mol in 1 µl). After 24 hours, no significant effect of KA application on IP accumulation was observed. However, 1 week following injection, significant increases of the metabotropic responses mediated by quisqualate (QX; 1µmol) and ibotenate (IBO; 1µmol) and trans-aminoacycloptene- dioxobutylate (t-ACPD) were noted in hippocampal slices. In control animals, the IP formation (IP synthesis) for buffer, QA, IBO, t-ACPD and carbachol were 28,028±491, 41,544±1051, 5,497±411, 7,508±5, 4,164±757 pmol/mg protein respectively whereas in KA treated animals they were 3,125±709, 11,071±1054, 13,196±228, 15,117±13, 5,664±119 pmol/mg protein. Significant increases were also obtained 2 and 6 weeks after injection. For carbachol, there was no significant increase at all times except after 6 weeks following KA treatment. This specific dramatic increase of the mGLU-R response in the hippocampus following seizure-related damage suggests that mGLU-R may play a role in the molecular mechanisms leading to brain plastic changes.


Glutamate receptors (GluR) are thought to play a role in epilepsy. We examined GluR gene expression in adult and 15 day rat brains after induction of status epilepticus by kainate (15 or 4.5 mg/kg i.p.). In situ hybridization was used to measure levels of NMDA1, GluR1, GluR2, and GluR3 mRNAs in corona sections from control and KA-injected animals 6 to 14 h after seizure onset. In adults, damage to amygdala, entorhinal cortex, and selective thalamic nuclei was evident at 6-12 h, while CA3/4 selective cell loss was not evident until 48 h. Autoradiography of adult brain sections indicated significant increases in GluR2 and GluR3 expression (50-100%) in the dentate gyms (DG) and subiculum (50%) and a comitant decrease (40-60%) in CA3/C4 hippocampal subfields at 24 h. GluR1 expression was unchanged. Emulsion-dipped sections revealed that the observed changes were in mRNA content per neuron. After 12 h adult NMDA1 mRNA levels were greatly reduced (CA3:60-80 %) but unchanged in DG suggesting a specific role for genomic expression of glutamate receptor subtypes in seizure activity. In 15 day pups, an age when KA does not induce neurodegeneration in limbic structures, GluR1 and GluR2 dentate mRNA levels were elevated in DG by 50-100% 24 h after seizure onset with no changes in GluR3 mRNA. The three transcripts were unchanged in CA3/C4. In conclusion, changes in adult mRNA expression occur prior to obvious CA3/4 pyramidal cell loss, but after afferent denervation from limbic structures. There are maturational differences in GluR mRNA expression that may be explained by the presence or absence of associated structural lesions.
412.5  CELL-ATTACHED RECORDINGS OF NMDA CHANNELS IN CHRONICALLY EPILEPTIC (KINDLED) NEURONS. G. Köhn* and I. Modéy. Center for Molecular Biology, Univ. of Heidelberg, F.R. Germany and Dept. of Neurology & Neuroscience, Univ. Sch. of Med. Stanford, CA.

Our studies in hippocampal slices and acutely dissociated neurons have shown that kindling-induced epilepsy increases synaptic activation of NMDA receptors. These changes are obtained in a whole cell, NMDA remained and alteration of the activation/inactivation kinetics of HVA Ca" currents. We have now examined if long-lasting changes can be detected at the level of single NMDA channels.

Cell-attached recordings were obtained in acutely dissociated control and kindled dentate gyrus granule cells in Mg-free medium containing 3 μM glycine using NMDA as an agonist (1, 5 and 10 μM). Single open times and the conductance of single channels were comparable in control and kindled neurons. Kindled channels differed from controls by having a longer burst duration and a higher +pwm at low NMDA concentrations (1 of 5 μM).

Following kindling, the combined effect of the longer burst duration and the higher +pwm was a 23-fold and a 3.6-fold increase in the charge carried through NMDA channels at 1 and 5 μM NMDA respectively. As a result of a reduced +pwm at 10 μM NMDA the charge through kindled channels was 57% of that recorded in control cells. On-cell dose-response studies, according to a double-fil method (A. Auerbach, Biophys. J., 60:660, 1991), were done by allowing NMDA (25 μM) to gradually diffuse to the channels over a period of 15:30 min. These experiments confirmed the higher affinity and augmented desensitization at high NMDA concentrations of kindled channels.

The chronic enhancement of NMDA receptor channel function may result from a covalent modification during kindling; the outcome for neuronal excitability conceivably contributes to epileptogenesis.

Supported by NINDS grant NS 12151 (I.M.) and a DFG Fellowship (G.K.).

412.7  DELAYED PHENCYCLIDINE EFFECTS ON NMDA SENSITIVE HGLUTAMATE BINDING X.-M. Gao* T. Kakati, C.A. Tannmisa, University of Maryland, MPRC, P.O. 21247; Baltimore, MD 21228.

Phencyclidine acts in a psychotomimetic way, which has a prolonged potomimetic effects in humans, hence it has been used as psychotomimetic model. We have previously demonstrated that single doses of PCP in rat produces an increase in +pwm than a prolonged decrease in cerebral glycose metabolism (rCMRGlu) lasting longer than 24 hours. This effect is prominent in rat limbic structures. Moreover, schizophrenia studies have shown a high degree of functional changes in limbic structures and postmortem pathologic changes in limbic and paralimbic regions. Therefore, we have begun to explore the neurochemical correlates of this delayed PCP action, especially in the limbic system. Here, we report the 24 hour effect of PCP (8.6 mg/kg) on the NMDA sensitive H-glutamate binding sites and other glutamate receptors. NMDA and AMPA glutamate receptors were quantified according to the method using H-glutamate and H-AMPA, respectively. Our results show an increase of more than 40% in NMDA binding in limbic structures like hippocampus, and in limbic related neocortex, like dorsolateral frontal, medial prefrontal, and posterior cingulate cortex, 24 hours after a single dose of PCP. This effect is significant in CA1 region of the hippocampus (dorsal: PCP=92.6±22.9, control=64.6±13.9 fmoles/mg tissue; ventral: PCP=86.0±15.0, control=62.8±11.8), Means±SD, n=6, P<0.05), and dorsolateral prefrontal cortex (PCP=52.0±16.3, control=26.8±5.6, P<0.01). No change in AMPA receptor density was found. These data suggest that the psychotomimetic effects of PCP, particularly of those delayed effects may be mediated by limbic glutamatergic synaptic selective for NMDA. These observations may suggest direction of study in schizophrenia.

412.9  Interactions of EtOH and Glycine with the NMDA Receptor-Linked Ion Channel Complex in EtOH Withdrawal Seizure-Prone and Resistant Mice and Rats. C.C. Crabb* and J.C. Crabbe. VAMC and Oregon Health Sciences Univ., Portland, OR 97201.

We have characterized the assay conditions that are required for equilibrium binding of [3H]MK-801 to the NMDA receptor-linked ion channel complex. Results of our experiments indicate that radioligand binding to a well washed tissue preparation (10 μM EtOH) includes glycine (10 μM) and NMDA (10 μM) requires 24 hrs to reach equilibrium. In addition, the dose response curve for the glycine-induced increase in the affinity of the binding sites is not altered after 24 hrs incubation at 25°C, with Hill coefficients increasing over time.

Under these conditions, EtOH (3 - 100 mM) has no effect on the characteristics or radioligand binding, on the glycine-induced increase in affinity for radioligand binding. In addition, there appears to be no difference in the density of [3H]MK-801 binding sites but there was a dose-dependent increase of EtOH withdrawal seizure-prone (WSP) and resistant (WSR) mice, which differ in handling-induced convulsion severity. Acute administration (24 hr inhalation) of the dose did not alter the density or affinity of binding sites in either mouse line. Thus, under well defined equilibrium binding conditions, EtOH does not directly interact with either the glycine or [3H]MK-801 binding sites on the NMDA receptor-linked ion channel complex. Differences between the effects of EtOH on radioligand binding under equilibrium and non-equilibrium conditions, and EtOH-induced altered behaviors will be discussed. This work was supported by NIAAA and the V.A.


Etanol is a selective inhibitor of the function of NMDA glutamate receptors. To assess changes in NMDA receptor function after chronic ethanol exposure, we measured NMDA-stimulated increases in intracellular Ca" in primary cultures of cerebellar granule cells. The cells were obtained from 8-day-old rats and exposed in vitro to 100 mM ethanol for two or more days or 30 mM ethanol for three or more days. In the ethanol-exposed cells, there was an increased maximal response to NMDA in the presence of glycine and glycine (in the presence of NMDA), with no change in the EC50 values. There was also no change in inhibition of the NMDA response by competitive or non-competitive antagonists or by ethanol added acutely. Chronic ethanol exposure apparently produced an increase in the number of NMDA receptors, with no change in their properties. Western blot analysis of membrane proteins using an antibody raised against the recently-cloned 70 kDa-glutamate-binding protein, believed to represent a subunit of an NMDA receptor, revealed a 50% increase in the amount of this protein in cells exposed to 100 mM ethanol for 2 days and a 30% increase for 4 days. These results are compatible with an ethanol-induced increase in synthesis, or decrease in degradation, of the glutamate binding subunit of an NMDA receptor. [Supported by grants AA 9005 and AA 4732 from NIAAA]
411.12 GLUTAMATE RECEPTOR SUBUNITS EXPRESSED IN XENOPUS OCYTES: SELECTIVE PLASTICITY IN HIPPOCAMPAL SYSTEMS:
University of Colorado, Boulder, CO 80309.

411.13 EXCITATORY AMINO ACID RECEPTORS IN SCHIZOPHRENIA:
SELECTIVE PLASTIC RESPONSES IN THE HIPPOCAMPUS OF
SOME SCHIZOPHRENIC INDIVIDUALS: I. Ulus*, L.C. Brunner, L.
Nugent and C.B. Salt. La Jolla, Calif. Research Unit in Brain Aging, University of
California, San Diego, CA 92121, USA.

411.14 SUBSTANTIA NIGRA NDMA AND AMPA RECEPTOR LOSSES
IN A MOUSE MODEL OF PARKINSON'S DISEASE:
U. Willner*, O. Isacson, J.B. Penney and A. B. Young.
Neurology Service, Massachusetts General Hospital, Boston, MA.

411.15 DEVELOPMENTAL CHANGE OF INHIBITION BY LEAD OF n-
METHYL-D-ASPARTATE-INDUCED CURRENTS IN CULTURED
HIPPOCAMPAL NEURONS: H. Ujihara, M. Alkon and E.K.
Baltimore, MD 21201.

411.16 THE SPASTIC HAN-WISTAR RAT AS A MODEL OF GLUTAMATE
EXCITOTOXICITY: ELECTROPHYSIOLOGICAL ANALYSIS. B.W. Cohran,
J.F. Marqués, J.B. Watton, N.A. Buchwald, and M.S. Levine.
Mental Retardation Res. Center, UCLA School of Medicine, Los Angeles, CA 90024.

Our laboratory has been studying a mutant strain of the Han-Wistar (HW) rat which
carries an autosomal, recessive gene causing spastic paraplegia characterized by ataxia,
tremor, and hind limb rigidity. Morphologically, starting about 30 days postnatally,
progressive cell death occurs in the Purkinje cell layer of the cerebellum and the CA3
layer of the hippocampus. Currently, we are investigating the hypothesis that
the cerebellar and hippocampal degeneration may be induced by glutamate (Glu)
excitotoxicity. Evidence was first obtained from Xenopus oocytes expressing HW rat
glutamate receptors following injection with mRNA from normal wild type (cont.
DNA injected controls). Oocytes injected with mutant HW cerebellar mRNA (45-50 days of age)
displayed significantly larger current responses to GLU and kainate (KA) than controls.
The dopamine in this disorder was studied by comparing mRNA of oocytes injected
with 32P-Glu and KA. These results showed a 40% increase in the maximum amplitude of the
response in the mutant cerebellum. This work suggests that enhanced presynaptic
release of glutamate is needed to evoke a response. These findings provide more evidence for abnormal,
glutamate neurotransmission in the cerebellum of HW rats which may underlie the
cellular degeneration observed in this mutation.
413.1

GABA A-receptors activate Ca 2+ entry into cultured spinal cord neurons via voltage-gated Ca 2+ channels (Wang et al., Neu. Abs. 17:977, 1991). Patch techniques were used to test directly if the activation of the GABA A-receptor depolarizes embryonic dorsal horn neurons and by what ionic mechanism. Membrane voltages responses to 10 μM muscimol, a GABA A-receptor agonist, were recorded in current-clamp mode using perforated patch recording. Pipettes contained (mM): K 2SO 4 75, KCl 100, HEPES 10 and 100 μg/ml tetrodotoxin (TTX) and 5 mM BATH. Next, 65 neurons tested during the first week in culture, 10 depolarized to between -30 and -40 mV, 26 to between -40 and -50 mV and 24 to less than -50 mV. 5 cells were hyperpolarized. To test the ionic basis of the depolarization, cells with depolarizing responses were identified, the perforated patch electrode was pulled out, then whole cell recording was established using a second pipette to control [Cl -]. Lowering [Cl -] to 40mM led to a depolarizing shift of +25 ± 2 mV (mean ± SEM; n=5) in the measured reversal potential. A +30 mV shift is predicted if Cl - is the only permeating extracellular anion. Reducing NaCl to 0.1 or 0.3 mM led to slightly depolarized reversal potentials (n=5) suggesting that Na + is not responsible for the depolarizing responses. These results demonstrate that GABA A-receptor activation can significantly depolarize a high percentage of embryonic cultured dorsal horn neurons and that an anionic conductance is mainly responsible for this effect, possibly due to a high [Cl -] (supported by EUKf and NIH).

413.2
GABA-INDUCED Ca 2+ TRANSIENTS IN TYPE 1 ASTROCYTES IN PRIMARY CULTURES. Michael Nilsson 1, Peter S. Eriksson 1, Lars Riemerks 1,2,3 and Elisabeth H. Hansson 1. 1Institute of Neurobiology and Department of Neurology, University of Göteborg, Göteborg, Sweden.

By using the Ca 2+-sensitive indicator fura-2/AM, the cytosolic Ca 2+ levels of type 1 astrocytes in rat cortical astroglial primary cultures, after stimulation with GABA, muscimol (GABA A–agonist) or baclofen (GABA B-agonist). We report that stimulation of both GABA A and GABA B receptors evokes Ca 2+ transient in type 1 astrocytes. In some cells, the responses after GABA stimulation were blocked to baseline levels after exposure to bicuculline (GABA A-antagonist). In other cells, bicuculline only slightly reduced the GABA-evoked responses, and the addition of phaclofen (GABA B-antagonist) did not amplify this inhibition. However, the muscimol-evoked rises in [Ca 2+] i were completely inhibited after exposure to bicuculline, while the responses after baclofen could only be partly blocked by phaclofen. GABA evoked rises in [Ca 2+] i lower than 50 μM; which alternatively were inhibited (mostly) or persisted in Ca 2+-free buffer. The rises in [Ca 2+] i persisted, but were reduced, in Ca 2+-free buffer after stimulation with muscimol, but were the contrary inhibited after baclofen stimulation. The results suggest that type 1 astrocytes in primary culture express GABA receptors which can elevate [Ca 2+] i directly or indirectly via Ca 2+ channels and/or via release from internal Ca 2+ stores.

413.3

In order to investigate the mechanism of action of Flupirtine, we tested its effect on cultured neurons from rat brain using the "whole-cell" configuration of the patch-clamp technique. The currents were measured using asymmetrical chloride concentrations, Flupirtine induced a dose-dependent, reversible depolarization. This was suppressed in the presence of bicuculline indicating that Flupirtine has GABA A-agonistic properties. With threshold concentrations of Flupirtine, the GABA-effect was enhanced in an over-additive manner. Experiments performed at voltage-clamped neurons demonstrated, that the action of Flupirtine is dependent on the membrane potential. At potentials more negative than -50 mV, the drug caused a monophasic chloride inward current. At more positive membrane potentials, the application of Flupirtine induced an additional transient outward current. Probing the effect with voltage-jump protocols showed, that this current is not carried by chloride, and thus is not mediated via the GABA A-receptor.

413.4

In previous work, stimulatory effects of diazepam (DZP) on free Ca 2+ were demonstrated using the membrane-permeant acetoxyethyl ester of the fluorescent dye fura-2 (fura-2/AM) in rat brain synaptosomes (Martín et al., 1991, Brain Res. 548:222). As an approach to evaluate the relative presynaptic and postsynaptic contributions to the observed effects of DZP on Ca 2+ metabolism, we compared the effects of DZP on Ca 2+ in synaptosomes and synaptic neuroneosomes from rat brain. A freshly dissected brain (without brainstem or cerebellum) was divided equally as starting material for the two preparations. Synaptosomes were made by the Percoll step gradient method of Dunkley, et al. (1986, Brain Res. 352:220). Both preparations were maintained in buffered salt solution in which NaCl was replaced by isomolar choline (136 mM choline chloride, 5.6 mM KCl, 1.3 mM MgCl 2, 11 mM glucose, pH 7.4) and incubated with fura-2/AM for 55 min, followed by extensive washing, so the only fluorescent response was due to fura-2 generated inside the organelles by endogenous esterases. In all cases, depolarization with 45 mM potassium increasing 1.2 mM CaCl 2 increased the level of cytoplasmic free Ca 2+. While DZP increased the levels of synaptosomal Ca 2+ in a dose-dependent fashion which was synergistic with the effects of depolarization (as before), there was no effect of DZP as high as 110 μM on synaptosomeroal Ca 2+. Since synaptosomeroal membranes are primarily post-synaptic in origin, these findings support the idea that effects of DZP on Ca 2+ may be selectively mediated (with a presynaptic mechanism. Supported by the Busch Fund and Rutgers University Research Council.)
413.5 EXCITOTOXIC LESIONS IN NEOSTRIATUM/GLORUS PALLIDUS CAUSE AN INCREASE IN IRON STAINING AND GLIAL CELL NUMBERS IN GLOBUS PALLIDUS AND SUBSTANTIA NIGRA. S. Sarty, G.W. Arnould, R. Richardson. Dept. of Biology and Institute for Biocomputational Science, University of Victoria, B.C., Canada, V8W 3P6.

The substantia nigra (SN) and globus pallidus, two iron-rich brain areas, receive a dense GABAergic innervation. This anatomic relationship suggests that GABA is important in iron metabolism. Therefore, we investigated the effects of iron accumulation after excitotoxic lesions of striatal/pallidal GABA inputs to globus pallidus/SN on iron staining and correlated histochemical and immunohistochemical markers with those results. Adult male Sprague-Dawley rats were divided into three groups. The first group received four intracerebroventricular infusions (5 μl of each) of saline; the second group received four infusions of bicuculline (10 μM); and the third group received four infusions of bicuculline (10 μM) and 6-OHDA (10 μM). Animals were sacrificed 1 week after surgery. The only lesion involved the substantia nigra/pallidus in the other two groups. Significant increases in iron staining and iron-positive cell counts were seen in the SN of the 6-OHDA lesions compared to saline- or bicuculline-injected rats. These results indicate that excitotoxic lesions compromising GABAergic innervation to SN and globus pallidus increase iron levels in areas, supporting a regulatory influence of GABA on brain iron.

413.7 GABA-INDUCED MEMBRANE CURRENTS IN DISSOCIATED MEDIAL SEPTUM/DIAGONAL BAND (MS/DB) NEURONS. D.R. Pytte and W.J. Griffith. Dept. of Med. Pharmacol. & Toxicol., Texas A&M Univ. Coll. of Medi- cine, College Station, TX 77843-1114.

Anatomic and electrophysiological data indicate that cells in MS/DB are GABAergic. Virtually all cells in these regions respond to GABA in a GABAergic synaptic contact. In the present study we present a significant fraction of spontaneous postsynaptic potentials recorded in vitro in MS/DB neurons. A large negative inactivation state (GABAA-induced membrane currents in all cells examined (rats, n=10; Guinea pigs, n=7)). Rapid application of GABA (100 μM) evoked a large negative inactivation state in all cells examined (50-100 μM). Whole-cell patch clamp recordings in acutely dissociated, adult MS/DB neurons identified GABA-induced concentration-dependent currents from a holding potential of -60 mV that reversed near 0 mV. Reponses to GABA (100 μM) significantly decreased in the presence of GABA (100 μM) which decreased an average of 42 ± 1.3% during the application. The effects of combined application of GABA with pentobarbital, midazolam or ethanol are currently being investigated. Supported by AA07005 (WHG); AA06322 and RD3A AA00101 (GDF).

413.9 MDA-RECEPTOR CHANNELS ARE SPECIFIC SOURCES IN THE SUPPRESSION OF GABA RECEPTOR FUNCTION BY INTRACELLULAR CALCIUM. N. Shi and A. Stolzen. Dept. of Pharmacology, SUNY Brooklyn, Brooklyn, NY 11203.

Elevation of intracellular calcium [Ca2+]i leads to a suppression of GABA receptors in freely dissociated CA1 pyramidal cells (cf. Chen et al., 1990, J. Physiol.). In the same preparation, we examined the contribution of various sources of [Ca2+]i elevating systems in the regulation of GABA receptors. Under whole-cell patch clamp recordings, two different calcium currents were elicited by voltage commands from -70 to +10 mV. One of these currents was blocked by calcium channel blockers. Alternatively, GABA responses were elicited by pressure application followed at similar intervals by GABA chloride current. GABA responses were measured at +10 mV under both experimental conditions. GABA responses generated through MDA-receptor channels were blocked by 200 μM bicuculline hydrochloride. In contrast to this, MDA-activated calcium currents were not blocked by bicuculline hydrochloride. Recovery from excitatory responses generated through MDA-receptor channels was slower than recovery from bicuculline hydrochloride-sensitive calcium currents. Therefore, it seems likely that two different types of calcium dependent GABA receptors exist. One type is calcium activated and the other type is calcium independent. The slower recovery from bicuculline hydrochloride-sensitive currents may be due to the presence of a calcium independent GABA receptor.


With the emergence of multiple GABA receptor subunit variants and many possible combinations in the brain, it has become a challenge to determine, from receptor binding studies, the number and pharmacological characteristics of the sites present. A major weakness has been the use of fairly nonselective radioligands. Critical to the determination of receptor heterogeneity is using curve-fitting programs such as LICAND, is the [H] of the radioligand must be determined at each putative site present, as the [H] of each competing ligand is dependent on this value. We have found that [3H]RO15-1788 is not sufficiently selective to clearly distinguish different receptor types using LICAND, however, its absolute nonselectivity cannot be assumed. We have been exploring a powerful new method to determine the Kd and receptor density of the ligand in several rat brain regions. This method, a Fourier-derived affinity spectrum analysis (FASA), (Anal. Biochem. 157(21), 1986), transforms receptor binding data (free and bound ligand conc.) into a probability-density function and solves it numerically, providing an affinity spectrum, as a function of affinity. No assumptions are made as to the number of binding populations. We have used this method to investigate the affinity of [3H]RO15-1788 at BDZ sites in several tissues, including the rat cerebellum and olfactory bulb. In each tissue, the radioligand [3H]RO15-1788 was competitively displaced from 0 to 20-fold. This information will greatly help the analysis of competitive binding data using LICAND, by determining the number of binding sites, and by providing initial and final guesses for the analysis. The use of the FASA method, along with data analysis using LICAND, should enhance our understanding of BDZ binding sites in the rat brain.

413.6 EFFECTS OF EXCITATORY AND INHIBITORY NEUROTRANSMITTERS ON WHOLE CELL RECORDINGS IN GUSTATORY ZONE OF NTS. L. Wang, M. S. King and R. M. Bradley. Dept. of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109.

The gustatory zone of the NTS consists of the lateral and terminals immunoreac- tive for substance P (SP) as well as GABA-ir neurons. Therefore, SP and GABA may have important influences on rostral NTS neurons. We investigated the effects of these transmitters on neurons recorded from the gustatory zone of the NTS using whole cell recording in slices of the rat medulla. Superoxions of Nm concentrations of SP transiently depolarized 54 of 88 neurons (61%) and increased input resistance, while GABA (10 μM) hyperpolarized 105 of 166 neurons (67%) and decreased input resistance. Since they could be elicited when synaptic transmission was blocked, these effects were due to direct postsynaptic action on the recorded neurons. GABA acted on both GABA and non-GABA neurons because both GABA (10 μM; GABA agonist) and bicuculline (10 μM; GABA antagonist) mimicked the effects of GABA. In addition, the GABA antagonist bicuculline (10 μM) and the GABA antagonist picrotoxin (10 μM) strongly suppressed the neuronal responses to GABA. Bicuculline also suppressed sympathetically in- duced IPSPs evoked by electrical stimulation of the solitary tract. Of 34 neurons tested, 15 (44%) responded to both SP and GABA, suggesting inhib- itory and excitatory modulation of single NTS neurons. The integra- tion of these excitatory and inhibitory influences may be important in process- ing gustatory information. This possibility is currently being investigated through the analysis of the effects of GABA and SP on single neurons in the gustatory zone of the NTS. [Supported by NHI DCOO28].

Modulation of GABA-mediated 36Cl influx into microsacs by ligands binding to the benzodiazepine (BDZ) site on GABA_A receptors is a potentially useful approach for the design of agonists and inverse agonists. To develop reliable conditions for assessing such actions, we have studied the effects of a prototypic agonist flunitrazepam (FLU 1μM) and inverse agonist DMCM (1μM) in rat cerebellar and cortical microsacs. In both tissues, most of GABA-mediated 36Cl influx occurred in the first 3 of 5s. Significant enhancement of this effect by FLU and reduction of it by DMCM was achieved in both tissues when the drugs were applied in the presence of 36Cl and GABA for 3s. The DMCM effect was in part due to a reduction of flux in the nominal absence of GABA. Although reproducible, the effects of these BDZ ligands are modest (-20%), and may be due to not all GABA mediated CI channels being sensitive to BDZs. To approach this problem, we have performed these studies in the presence of zinc (100μM), known to preferentially inhibit GABA_A receptors that are insensitive to BDZs because they lack a γ subunit. The presence of Zn substantially reduced GABA (100μM) mediated 36Cl flux in cortex (61% and) in cerebellum (51%+) suggesting a significant proportion of the CI channels are likely insensitive to BDZs. Under these conditions FLU increased dramatically to 273% from 42% without Zn. These results indicate that optimum conditions for measuring the effects of BDZ agonists and inverse agonists on GABA mediated 36Cl flux involves measuring the effect within 3s of addition of GABA, but with the ligands in prior contact with the GABA receptor. Addition of Zn appears to enhance the effect of these ligands by allowing the measurement of CI flux only through BDZ sensitive GABA channels.

3. MSL 26479 MEDULATES GABA RECEPTOR ACTIVATION: SINGLE CHANNEL CURRENTS FROM CULTURED RAT HIPPOCAMPAL NEURONS. C.J. Rogers and K.M. Giesler, Marion Merrell Dow Research Institute, Cincinnati, OH 45215.

GABA receptor complex chloride current is gated through the integral ion channel by GABA, receptor activation and mediated via allosteric receptor interactions. MSL 26479, may act via a benzodiazepine-like binding site as an inverse agonist. In vivo, MSL 26479 displaced [3H] Ro 15-1788 binding consistent with a benzodiazepine-like interaction. Extracellular recordings from the hippocampal slice enabled enhanced sensitivity to MSL 26479. [3H]-butyrylcholine-3 binding was increased with MSL 26479 suggesting a benzodiazepine-like agonist-like action. Behavioral studies indicate MSL 26479 enhanced respiration. To determine whether MSL 26479 modulates GABA-activated CI current and the mechanism by which it may do so, we used the single channel patch clamp recording technique. Single channel recordings were obtained from cultured rat hippocampal axons in response to the positive application, from 1-5μs across diameter accessibility, of GABA (2μM) alone or GABA (2μM) plus MSL 26479 (10, 100, or 300μM). Single channel currents were pooled from up to 80 different recordings for each of the different conditions tested. MSL 26479 modulated GABA-activated CI current in a concentration dependent manner. Total CI current, comparing GABA (2μM) alone versus GABA (2μM) plus MSL 26479 (12μM) was decreased by 38%, decreased by 49% at 120μM, but increased by 134% at 300μM. The mean open duration of the channel for the 30 pS conductance state was concentration dependent: 2.42 μs, GABA (2μM) (6.3 μs, GABA (2μM) plus MSL 26479 (2μM)); 3.16 μs, GABA (2μM) plus MSL 26479 (10μM), and 1.47 μs, for GABA (2μM) plus MSL 26479 (300μM). Further investigations are underway to better understand this concentration dependent response and to determine whether this compound selectively activates specific receptor subunit configurations.

4. A COMPARISON OF THE ACTIONS OF THE BENZODIAZEPINE (BZ) PARTIAL AGONIST, Ro16-6208, WITH "FULL AGONIST" BZs ON THE GABA_A RECEPTOR COMPLEX (GRC). D.A. Finta and K.V.G. Dept. Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92717.

Ro16-6208 (Bretazenil) is a relatively new imidazo-diazepine derivative with a pharmacological profile characteristic of a BZ partial agonist. Ro16-6208 exhibits potent anticonvulsant and anticonvulsant effects with minimal psychomotor impairment or development of physical dependence. The present study utilized modulation of [35S]-butyrylcholine binding and enhancement of GABA-stimulated 36Cl- (chloride) uptake as measures of GRC function to further assess Ro16-6208's partial agonist profile. Ro16-6208 was the most potent of the examined compounds (concentration at which half-maximal inhibition of specific [35S]- butyrylcholine binding occurs) of 1.1mM, compared to 2.7mM for diazepam (DZP), 4.3mM for clonazepam (CLON) and 47.4mM for flunitrazepam (FLU). However, Ro16-6208 was less efficacious in that it produced 27% inhibition of specific [35S]- butyrylcholine binding, compared to DZP (49%), CLON (34%) and FLU (41%). In the presence of 3mM DZP, Ro16-6208 antagonized the inhibition of DZP alone. These data provide further support that Ro16-6208 is acting as a partial agonist at the BZ receptor in modulating [35S]- butyrylcholine binding. (Supported by NIH grants NS25986 and NS24645).


A range of biochemical markers of GABAergic and dopaminergic (DAergic) neuronal activity were examined in the CNS of Roman high-avoidance (RHA/Ver) and Roman low-avoidance (RLA/Ver) rats. The dopamine transporter (DAT) was measured in the striatum and the nucleus accumbens in RHA/Ver and RLA/Ver. RHA/Ver rats had a lower DAT density than the corresponding RLA/Ver rats. In addition, the dopamine level in the nucleus accumbens of RHA/Ver rats was lower than that of RLA/Ver. A similar, but less marked, difference was observed in the ventral pallidum. The serotonin transporter (SERT) was measured in the nucleus accumbens and the raphe nuclei. RHA/Ver rats had a lower SERT density than RLA/Ver rats. In addition, the serotonin level in the nucleus accumbens of RHA/Ver rats was lower than that of RLA/Ver. A similar, but less marked, difference was observed in the ventral pallidum. The binding affinity of [3H]-GABA to the GABA_A receptor was measured in the striatum and the nucleus accumbens in RHA/Ver and RLA/Ver rats. RHA/Ver rats had a lower binding affinity than RLA/Ver rats. In addition, the GABA level in the nucleus accumbens of RHA/Ver rats was lower than that of RLA/Ver. A similar, but less marked, difference was observed in the ventral pallidum. The binding affinity of [3H]-flunitrazepam to the GABA_A receptor was measured in the striatum and the nucleus accumbens in RHA/Ver and RLA/Ver rats. RHA/Ver rats had a lower binding affinity than RLA/Ver rats. In addition, the flunitrazepam level in the nucleus accumbens of RHA/Ver rats was lower than that of RLA/Ver. A similar, but less marked, difference was observed in the ventral pallidum. The binding affinity of [3H]-35s-TP5 to the GABA_A receptor was measured in the striatum and the nucleus accumbens in RHA/Ver and RLA/Ver rats. RHA/Ver rats had a lower binding affinity than RLA/Ver rats. In addition, the 35s-TP5 level in the nucleus accumbens of RHA/Ver rats was lower than that of RLA/Ver. A similar, but less marked, difference was observed in the ventral pallidum. The binding affinity of [3H]-DAMGO to the opioid receptor was measured in the striatum and the nucleus accumbens in RHA/Ver and RLA/Ver rats. RHA/Ver rats had a lower binding affinity than RLA/Ver rats. In addition, the DAMGO level in the nucleus accumbens of RHA/Ver rats was lower than that of RLA/Ver. A similar, but less marked, difference was observed in the ventral pallidum. The binding affinity of [3H]-naloxone to the opioid receptor was measured in the striatum and the nucleus accumbens in RHA/Ver and RLA/Ver rats. RHA/Ver rats had a lower binding affinity than RLA/Ver rats. In addition, the naloxone level in the nucleus accumbens of RHA/Ver rats was lower than that of RLA/Ver. A similar, but less marked, difference was observed in the ventral pallidum. The binding affinity of [3H]-dextrorphan to the opioid receptor was measured in the striatum and the nucleus accumbens in RHA/Ver and RLA/Ver rats. RHA/Ver rats had a lower binding affinity than RLA/Ver rats. In addition, the dextrorphan level in the nucleus accumbens of RHA/Ver rats was lower than that of RLA/Ver. A similar, but less marked, difference was observed in the ventral pallidum.
414.1

A cis ELEMENT IN THE NEUROPEPTIDE Y GENE MEDIATES STIMULATION BY NERVE GROWTH FACTOR

Neuropeptide Y (NPY) gene expression, in rat PC12 cells, is stimulated by nerve growth factor (NGF) at the level of transcription. To investigate the mechanism by which NPY expression is enhanced by NGF, we report an NGF-responsive, cis-acting sequence, the NGF response element (NRE).

414.2

TRANSCRIPTIONAL CONTROL OF HIPPOCAMPAL NEUROPEPTIDE Y EXPRESSION

Hippocampal neuropeptide Y (NPY) expression is predominantly localized to neurons in the dentate gyrus hilus and stratum oriens. In the current study, we identified the affected promoters by transfection experiments with and without NPY expression in the absence of NGF. We report that the NPY expression is increased by NGF in the presence of NGF, but not in the absence of NGF.

414.3

SECOND MESSENGER AND RECEPTOR-MEDIATED REGULATION OF SUPERIOR CERVICAL GANGLION NEUROPEPTIDE Y (NPY) EXPRESSION AND SECRETION, V. Mys and K.M. Bras, Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Superior cervical ganglion (SCG) neurons synthesize and secrete calcitonin gene-related peptide. We have learned that calcitonin gene-related peptide is released in response to a variety of stimuli. We have studied the regulation of neuropeptide Y (NPY) expression and secretion in primary rat SCG neuronal cell cultures. Enzymatically dissociated SCG cells from newborn rats were treated with 10 μM cAMP or 10 μM cGMP to increase the intracellular concentration of calcium.

414.4


Gene expression of neuropeptide Y (NPY), an important neurotransmitter and neuromodulator, is regulated by neural activity via neural activity by transduction of intracellular signals. The effect of neural activity on NPY gene expression was measured by a new method that uses calcium imaging and transduction of intracellular signals. The effect of neural activity on NPY gene expression was measured by a new method that uses calcium imaging and transduction of intracellular signals. The effect of neural activity on NPY gene expression was measured by a new method that uses calcium imaging and transduction of intracellular signals. The effect of neural activity on NPY gene expression was measured by a new method that uses calcium imaging and transduction of intracellular signals. The effect of neural activity on NPY gene expression was measured by a new method that uses calcium imaging and transduction of intracellular signals.
414.5 NEUROPEPTIDE Y EXPRESSION IN TARGET SPECIFIC NEURONS IN THE RAT SUPERIOR CERVICAL GANGLION. A.E. Elsheikh, K. Hentschel, and L.L. Wright, Dept. Anatomy/Neurobiology, BUSHM, Boston, MA 02118.
This study was to compare the pattern of neuropeptide Y-like immunoreactivity (NPY-ll) in adult and developing rat sympathetic superior cervical ganglion (SCG) neurons projecting to purely vascular targets, and those projecting to "mixed" vascular and glial elements. Adult and postnatal day 1 (P1), P5, P25, and P60 rats were used. The retrograde tracer Fluorol Gold (FG) was injected into the temporals muscle or frontal cortex to label SCG neurons projecting to a "purely vascular" target, and into the submandibular gland, to label neurons projecting to a "mixed" target. In each case, FG was utilized to compare the relative proportions of each population of SCG neurons that contained NPY-ll. Analysis of the results showed that nearly all (98%) of temporals projecting neurons contain NPY, most (76%) of cerebral blood vessels projecting neurons contain NPY, and few (9%) of submandibular projecting neurons contain NPY. The results were similar at P5, P15, and P25, in that most of the SCG neurons projecting to the cerebral blood vessels and temporals muscle contained NPY-ll, while significantly few of those projecting to the submandibular gland contained NPY-ll. From these data, we conclude that the population of neurons projecting to these target organs differs significantly in the percentage of neurons displaying NPY-ll in adult and that this pattern is present after the fifth postnatal day of development. This work has been supported by a B.U. GSRA to AFE.

The neuronal localization of vasopressin (VP) mRNA was carried out using digoxigenin-labeled oligonucleotide complementary DNA probes 908-943 of the VP cDNA. Vibratome sections of rat brain fixed by perfusion with 4% paraformaldehyde and 0.1% glutaraldehyde were processed for in situ hybridization or with triton X-100 and hybridized with the digoxigenin-labeled probe. Following the washing steps, the digoxigenin was detected using an anti-digoxigenin antibody and the ABC-peroxidase method. The peroxidase was revealed with DAB enhanced with NiCl2, the sections were postfixed with osmium tetroxide and embedded in epoxy resin. This method allowed us to label the perikarya of magnocellular neurons of the rat hypothalamus. In addition, its high sensitivity was demonstrated by the labeling of VP neurons of the suprachiasmatic nucleus, where VP mRNA concentration is known to be low. At the electron microscopic level, the well preserved morphology allowed for precise subcellular localization of VP mRNA. 1. MAGNOCellular PERIKARYA. In the perikarya, the label was mainly associated with the membrane of the rough endoplasmic reticulum (RER), but not its lumen. This labeling was localized to discrete areas along the RER, suggesting a compartmentalization of VP mRNA within the RER. The Golgi apparatus, as well as neurosecretory granules appeared to be devoid of label. 2. AXONAL COMPARTMENT. Some axonal swellings in the median eminence, containing secretory granules, were highly labeled. These positive swellings were more abundant in salt-loaded rats. Some positive swellings were also observed in lactating rats and much more rarely in control animals. Within these swellings, the labeling was diffuse and therefore the precise localization of VP mRNA could not be ascertained. This limitation may be overcome through the use of colloidal gold probes.

414.7 ARGININE VASOPRESSIN (AVP) GENE EXPRESSION IN DISCRETE AREAS OF THE RAT BRAIN FOLLOWING PRE-TREATMENT WITH AVP. P. Pouliot, P. Scoy, D.J. Pitman and D.M. Dorog, GREC, VA Medical Center, Seattle, WA 98108.
It has been shown that AVP enhances its own release and enhances (sensitizes) the responses of the rat brain to subsequent AVP exposure. One possible mechanism for these effects may be by AVP-induced alteration in AVP gene expression. The level of cytoplasmic AVP mRNA was quantified autoradiographically in discrete areas of male Sprague Dawley rat brain 24 hours following AVP (100 pmol iv) or vehicle (physiological saline iv) pretreatment. Following decapsulation, brains were frozen and 20 pm sections were hybridized with a [35S]labeled probe for AVP mRNA, emulsion coated, developed and analyzed for the number of labeled cells and/or for dotdensity. Preliminary results show that while AVP pretreatment enhanced the number of hybridization positive neurons in the Bed nucleus of the stria terminalis increases from 62 (control) to 35 (AVP pre-treated), the number of AVP mRNA expressing cells in the mediodorsal (16 control; 18 AVP pre-treated) and the paramentric nuclear (rod. 284 control; 283 AVP pre-treated) was not enhanced by AVP pre-treatment. These results suggest that pre-exposure of the rat brain to AVP results in discrete alterations of the expression of AVP messages. Studies are now in progress to evaluate AVP mRNA levels in discrete areas of rat brain 1, 3, 6, and 12 hrs following AVP (100 pmol iv) or vehicle (physiological saline) pre-treatment. This work was supported by Savoy Foundation (PF) MRC (OJP) and VA medical center and NS 20311 (PS and DMD).

414.8 CCK mRNA EXPRESSION, PRO-CCK PROCESSING AND REGULATED SECRETION OF CCK IMMUNOREACTIVE PEPTIDES BY THREE ENDOCONE TUMOR CELLS IN CULTURE: A VALID MODEL FOR BRAIN CCK?
M.C. Benoit-Deloze, Dept. Pharm. & Physiol. Sci., St. Louis Univ. School of Medicine, St. Louis MO 63104.
Subclones of three common endocrine cell lines At-T20, rat insulinoma (RIN 5f) and a thyroid medullary carcinoma, both express a single CCK mRNA species the same size as that found in rat brain. The WE cells contained levels of immunoreactive CCK comparable to rat cerebral cortex (about 4 ng/ mg protein). While the other cell lines contained significantly less CCK. Like the rat brain, they were able to correctly process pro-CCK to a form which co-eluted with CCK 8 on Sephadex and HPLC. All three cell lines secreted CCK as well as CCK 33, and this secretion was significantly enhanced by cAMP, but not by tumor-promoting phorbole esters. When transfected with a eukaryotic expression vector containing the rat CCK cDNA behind the CMV promotor, the At-T20 cells expressed about 500 times as much CCK as the wild type. These cells still correctly processed pro-CCK to CCK 8, which suggests that either the expression of the processing enzymes is coordinately regulated with expression of the proenzyme or that the enzymes are present in a considerable excess. In summary, these cell lines appear to be a good model for studying pro-CCK processing in the brain and should facilitate future studies of CCK expression, biosynthesis, processing, and secretion. Supported by NIH 18667.

414.9 HALOPERIDOL-INDUCED NEUROTENIN GENE EXPRESSION IN STRIATAL NEURONS IS REDUCED BY CHRONIC TREATMENT. R.S. Merchant, D.J. Dobie and D.M. Dorog, Grec, VA Medical Center, Dept. of Psychiatry & Behavioral Sciences and Pharmacol., Univ. of Washington, Seattle, WA 98195.
Expression of neurotensin/neuromedin N (NT/N) gene in dorsolateral striatal (DLS) neurons appears to be under the regulation of dopamine D2 receptors such that acute blockade of D2 receptors by neuroleptics (e.g., haloperidol) raises the level of NT/N mRNA by 1000%. The present study investigated the responses of the NT system to chronic haloperidol administration, a treatment which has been shown to attenuate the expression of D2 receptors. Male Sprague Dawley rats were treated s.c. with saline or haloperidol (1 mg/kg/d) using osmotic minipumps and sacrificed on day 28. A separate group of rats was treated similarly followed by removal of the pumps on day 28 and was challenged 24 hr later with a single dose of saline or haloperidol. Expression of NT/N mRNA was examined by in situ hybridization. Neuronal mRNA localization was revealed using NT/N mRNA expression in DLS was significantly lower in rats which received chronic haloperidol when compared to those which received acute haloperidol following chronic saline treatment. The tolerance in NT/N mRNA expression was not modified by an acute haloperidol challenge to chronically treated with haloperidol. It is likely that the decreased sensitivity of NT/N cells in the presence of haloperidol reflects a compensatory post synaptic response to upregulation of D2 receptors. (Supported by Scottish Rite Schizophrenia Foundation, the VA, and NS 20311)

414.10 REGULATION OF SOMATOSTATIN GENE EXPRESSION IN DISOCIATED CULTURES FROM CEREBRAL CORTEX. G.L. Coopers, and C.B. Choi, Dept of Pediatrics, The Kennedy Krieger Institute and Johns Hopkins Medical Institutions, Baltimore MD 21205
Somatostatin (SOM) is a small peptide transmitter which is found in the cerebral cortex. The two biologically active forms SOM-14 and SOM-28 are both derived from the same inactive precursor molecule preprosomatostatin (pPSOM). Within the cortex, SOM is localized to a small subset of neurons and comprises about 2% of the total neuronal population. These neurons have been implicated in the pathogenesis of a variety of diseases (Down's syndrome, Epilepsy and hypoxic-ischemic encephalopathy). Previous studies have demonstrated that the pPSOM gene can be regulated by such diverse stimuli as second messengers (cAMP, neurotransmitters, electric field) and other factors. To further study the effects of cAMP, primary cultures of dissociated cerebral cortex were made from E15 mouse brain and grown for 7 days in MEM, with 10%FCS/10%HS in 95% air and 5% CO2. Cultures were stimulated with cAMP (2.5 x 10-5 M) at days 3 and 10. All cultures were stimulated with cAMP at day 10. At day 10, total RNA was isolated at 4,8,12 and 24 hours. Changes in pPSOM mRNA abundance was measured using Northern blot analysis. Stimulation with 2.5 x 10-5 M cAMP resulted in an (10-fold) increase in pPSOM mRNA by 4 hours and a (50- fold) increase by 8 hours. When cultures were stimulated with BcAMP increased pPSOM mRNA was also seen at 4 hours (2-fold), 8 hours (5-fold), 12 hours (9-fold), 24 hours (7-fold) compared to unstimulated controls. Our results support previous findings which demonstrate that pPSOM gene expression is regulated via cAMP mediated signal transduction pathway and extends the observation to neurons in the developing cerebral cortex. Supported by CIDA 1-K08-NS01466-01A1.

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Somatostatin (SRIF), a neuropeptide produced by C cells in the thyroid gland, has been implicated in the modulation of thyroid hormone function. In the present study, we have assessed whether SRIF gene regulation is regulated by calcium or triiodothyronine (T3). Since cyclic AMP is a known modulator of SRIF secretion, we also evaluated the effect of forskolin or a cyclic AMP analog on SRIF production. Rat medullary thyroid carcinoma cells (MET-6-23) were grown in DMEM and 15% horse serum, and serum-deprived cells were incubated in DMEM 0.05 mg/ml heparin. The media content of SRIF and calcitonin was determined by radioimmunoassay. Northern blot analysis (20 μg total RNA) was used to assess SRIF mRNA levels. Forskolin (10 μM) or the cyclic AMP analog (10 μM) increased SRIF mRNA levels and SRIF as measured by intracellular SRIF mRNA levels (p<0.05 vs. 0.5 μM calcium). However, T3 (10 μg/mL) had no effect on SRIF mRNA levels or media content. These results indicate that calcium acts to increase calcitonin release as well as cause increases in both SRIF secretion and steady state SRIF mRNA levels. Since SRIF has been shown to inhibit calcitonin secretion, SRIF may act in an autocrine fashion to limit calcitonin production in response to a calcium challenge. (Supported by WVU Med.Corp. and NIH BRG No.2.507 RR05433-29).

414.13 DOPAMINERGIC REGULATION OF TRANSCRIPTIONAL ACTIVITY OF ENKEPHALIN GENE IN RAT BASAL GANGLIA. S. P. Swain and A. L. Chaudhari. Department of Pharmacology & Toxicology, Indiana University School of Medicine, 3400 Broadway, IN 46202.

This study examined whether a deficiency of dopamine during neonatal developmental period will induce permanent alterations in the rate of transcription of enkephalin gene. Dopamine deficiency was induced in Sprague-Dawley rat pups, by intracerebral administration of 100 μg of free base of 6-hydroxydopamine (6-OHDA) on the third day after birth. The animals were sacrificed 60 days after the 6-OHDA lesion. Striatal tissues were used for the determination of preproenkephalin (PPE) mRNA by Northern blot analysis, Met5-enkephalin (ME) levels by radioimmunoassay and amines and metabolites by HPLC. The rate of transcription of PPE gene was determined by a nuclear run-on assay using nuclei isolated from fresh or frozen tissues; the 32P-labeled run-on transcripts obtained from a run-on transcription reaction were hybridized to a slot-blotted nitrocellulose or nylon membrane containing a plasmid with rat cDNA for PPE. As expected, dopamine-denervated animals exhibited a significant loss of dopamine (>90% of control), an increase in PPE-mRNA and an increase in ME levels. The rate of transcription of PPE gene as evidenced from the run-on transcription assay was increased in lesioned animals. The increase in the rate of PPE transcription gene coupled with an increase in PPE-mRNA and its translation product ME indicate an enhanced rate of biosynthesis of enkephalin in lesioned animals. The results suggest a crucial role for dopamine in the development and regulation of enkephalin neurons of the basal ganglia. Supported by USPS grant NS29603.


Dexamethasone (DEX, 10^-7 M) increased [Met5]enkephalin (ME) secretion from cultured striatal and hippocampal cells 2-fold over basal release after 24 hrs and 4 days exposure, an effect comparable to stimulations with the adenylate cyclase activator forskolin (FSK, 10^-5 M) and the protein kinase C activator phorbol myristate acetate (PMA, 10^-7 M). DEX strongly potentiated the stimulation by FSK (to > 8-fold over basal) at 4 days, but not after 24 hrs. DEX did not increase the response to PMA or to the Ca2+ ionophore ionomycin (10^-6 M). The potentiation of DEX was mimicked by corticosterone and competitively blocked by the anti-glucocorticoid RU486. Surprisingly, progesterone pretreatment abolished the DEX effect. Stimulated ME release at 4 days reflected synthesis of peptide, being much greater than cell ME content, and increased preproenkephalin (PPE) mRNA was observed in situ hybridization studies. In hippocampal cultures, PPE mRNA and ME and precursor peptides appeared restricted to a subpopulation of type-I astrocytes. In striatal cultures, neurons may also contribute to the observed effects of DEX on ME.


We have previously reported that long-term exposure of BAM cells to nicotine increases both the secretion of ME and the expression of proENK gene. In order to elucidate the molecular mechanisms underlying these long-term effects of nicotine, studies were undertaken to determine whether continuous activation of nicotinic receptors was required for both the delayed increase in ME secretion and proENK mRNA. Cholinergic receptor antagonists, hexamethonium (1 mM) and atropine (0.5 μM), were added to the incubation media at different time points (0.5, 1, 3, 6, 9, 12, and 24 hr) after nicotine treatment and aliquots of incubation medium were taken at each time point for ME determination and the cells were washed and incubated 24 hr for proENK mRNA measurements. Nicotine (10 μM) stimulated both distinct phases of ME secretion; a short-term rapid phase, followed after 9 hr by a sustained increase in ME release. Hexamethonium and atropine added 0.5 to 6 hr after nicotine treatment significantly inhibited the long-term phase of ME secretion and the expression of the proENK mRNA levels measured after 24 hr of nicotine treatment. Unexpectedly, these antagonists were ineffective in blocking the nicotine-induced responses when added 9 hr before treatment. Nuclear run-on assays showed that the transcriptional rate for proENK mRNA was increased by nicotine for at least 9 hr after drug treatment. Our results suggest that the long-term (at least 9 hr) nicotine effect on ME secretion and proENK mRNA is mediated by the long-term stimulation of the ME increase in proENK mRNA levels induced by nicotine reflects an increased transcription of the proENK gene. Currently, we are comparing these effects of nicotine with effects of this drug on the expression of tyrosine hydroxylase mRNA and the short- and long-term secretion of catecholamines.


The promoter region of the preproenkephalin gene contains elements for regulation by a number of transcription factors and second messenger systems. In striatum and glia, cAMP and CREB appear important for regulating ME. In rat adrenal, there is evidence for AP-1 binding as a primary regulator, and in spleen NFκB has been reported to regulate enkephalin expression. Nicotine stimulates adrenal medulla and sympathetic and parasympathetic ganglia. Chronic nicotine (3mg/kg bidally) was no more effective than saline injections at increasing adrenal ME in agreement with reported findings. However, acute repeated nicotine injections (3mg/kg every 30 minutes for 3 hours) produced a significant 2-fold increase relative to saline injections when measured after 2 days. Adrenal medulla AP-1 binding was decreased by nicotine and the decrease was long lasting (12 hrs) after acute repeated injections. Adrenal NFκB binding was increased by nicotine. In spleen, repeated acute nicotine injections also increased ME by about 2-fold after 2 days. Both AP-1 binding and NFκB binding were increased in spleen by nicotine. Our data indicate differential regulation of ME in peripheral tissues by nicotine.
414.17

EFFECT OF NICOTINE ON RELEASE OF CALCITONIN GENE-RELATED PEPTIDE FROM THE RAT TRACHEA

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Calcitonin gene-related peptide (CGRP) exists in the peripheral terminals of sensory C-fibers which innervate the airway. Topical nicotine is known to stimulate these terminals. Given this excitatory effect, the present study assessed whether nicotine could evoke the release of C-afferent CGRP from the rat trachea. The rat trachea (from large to carina) was dissected free and incubated in oxygenated Krebs solution kept at 37°C. After 30 min equilibration time, the trachea was sequentially incubated in tubes containing 2 ml of: 1) Krebs (baseline); 2) Krebs + drugs (nicotine or capsaicin); and 3) Krebs (control) for 10 min. CGRP levels in each test tube was analyzed by RIA. Nicotine (5x10⁻⁶ to 5x10⁻⁵M) caused a concentration-dependent increase in the levels of CGRP in the perfusate. Forty minutes after the first exposure to nicotine, a second challenge with nicotine revealed a concentration-dependent desensitization of CGRP release. The releasing effect of capsaicin (10⁻⁴M) was unaffected by nicotine desensitization. Pretreatment, however, with capsaicin (10⁻⁴M) blocked nicotine (10⁻⁵M)-induced peptide release. The result suggests that nicotine activated the peripheral terminals of capsaicin-sensitive primary afferents in the trachea and released CGRP.


This work is supported by Tobacco Related Disease Program, University of California. X.-H. Y.

414.2

CYSTEAMINE PRETREATMENT ENHANCES INHIBITORY ACTION OF S₈S IN THE DENTATE GYRUS

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We have previously reported that both S₈S and S₈S₄ act as an inhibitory action on the evoked ECFP pop spike recorded from the dentate gyrus (DG) and cause direct postsynaptic hyperpolarization of dentate granule cells. These experiments were designed to examine the effects of the somatostatin-depleting agent cytoxamine on responses to S₈S and S₈S₄ in DG. 500 µm thick transverse rat hippocampal (HPC) slices were completely submerged and continuously superfused in a recording chamber with oxygenated pH buffered ACSF. Three to four week old Sprague Dawley rats were pretreated with a single injection of 200 µg/kg of cytoxamine given 15 to 20 hours prior to preparation of brain slices. All recordings were carried out in 10⁻⁴ M bicuculline. Cysteamine pretreatment resulted in selective increase in the inhibitory action of S₈S₄ on the DG ECFP. Threshold of inhibitory effect was noted at 0.1 µM with complete and reversible suppression of evoked activity being observed at concentrations of 3 to 5 µM. Interestingly, inhibitory action of S₈S was reduced in the cytoxamine pretreated slices. When cytoxamine was added to the perfusate (100 nM) a gradual increase in amplitude and number of evoked pop spikes was observed and this increase in activity persisted during the wash. These findings indicate that cytoxamine pretreatment results in selective enhancement of the response to S₈S with relative down regulation of S₈S₄ response and suggests the possibility of a differential effect on receptor subtypes. The increased amplitude and repetitive firing observed during bath application of cytoxamine supports the concept that somatostatinergic neurons in the DG play an inhibitory role in controlling the firing of DG granule cells and that loss of these neurons may be involved in the pathophysiology of certain seizure disorders.

414.3

KEENOPROTECTIVE AND LTP-ENHANCING EFFECTS OF CHOLESTORBININ 18 Hippocampal slices, K. Yasui, M. Fushiki, Y. Nakazawa, W. Li, H. Shinya, A. Ando, and T. Araki, Kinki Medical College, Osaka, Japan

Cholescolystokinin octapeptide (CCK-8) is densely distributed in the hippocampus, however its functional significance in this area is still unclear. Here we demonstrate neuroprotective and long-term potentiation (LTP)-enhancing effects of CCK-8. CCK-8 dose-dependently enhanced the magnitude of LTP induced by tetanic stimulation of Schaffer collateral-axosomatic synapses in the CA1 region of guinea pig hippocampal slices. This enhancing effect was antagonized by pretreatment of CCK-8 receptor antagonist. Furthermore, desulfated CCK-8 showed a similar action on the LTP. The late negative component of evoked response, which was induced by a K⁺ channel blocker and by elevation of extracellular K⁺, was reduced by CCK-8. In vitro ischemic insult (hypoxia & hypoglycemia) induced a transient disappearance of evoked response followed by a short hyperexcitability period and a long-lasting dysfunction of CA1 pyramidal neurons in hippocampal slices of stroke-prone spontaneously hypertensive rat. CCK-8 reduced spreading depression-like depolarization and shortened time for the early disappearance of evoked potential as well as that for recovery of population spike. This neuroprotective action was also mediated by CCK-8 receptors. These results suggest that CCK-8 plays a positive role in the muscle system and shows neuro-protective effects possibly by closing K⁺ channels via breakdown of phosphoinositide in the hippocampus.

414.4

VASOPRESSIN ELICITS A LASTING INCREASE IN EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPs) IN VENTRAL HIPPOCAMPUS NEURONS, J. L. Urban and P. L. French, Rudolf Magnus Institute, University of Utrecht, The Netherlands.

(Vanop: EDA)

Vasopressin (VP) is a neuropeptide which is synaptically released in several brain structures, including the lateral septum (LS) and ventral hippocampus (VH). Small amounts of VP, applied centrally, altered acquisition and extinction of various conditioned behaviors as well as the activity of brainstem. In LS slices, pH concentrations of VP for hours enhanced EPSPs in LS neurons, without affecting the resting membrane potential (RMP), input resistance (IR) and other properties of these neurons. We used conventional microdialysis (3M KAc) and studied in brain slices the effect of VP on the EPSPs in neurons in the CA1/subiculum region of VH, evoked by stimulation of the stratum radiatum. Application for 15 min with 10⁻⁴ M VP had little effect on either RMP (before VP: 60.67±5.7 mV; 35 min after VP: 60.73±5.3 mV), IR (before VP: 46.84±6.5 MΩ; 35 min after VP: 49.28±7.4 MΩ) or the threshold for action potential was little changed by VP (before VP: 50.5±3.4 mV; 35 min after VP: 48.76±4.9 mV). In 14 of 20 neurons studied, EPSPs commenced to increase during and after VP application. At 35 min after the peptide application the amplitude of the EPSPs obtained on average 137.5% of the control values and remained at this level for the rest of the recording (max 2.5 hr). In the remaining 6 neurons EPSPs either did not changed (n=4) or decreased (n=2) after the VP treatment. Thus, VP concentrations of 10⁻⁴ M can for many hours enhance the excitability of the excitatory, presumably glutamatergic, transmission in CA1/subiculum neurons of the VH presumably by augmenting the glutamate release and/or the sensitivity of postsynaptic glutamate receptors. This VP effect was presumably different from the short excitatory action on the hippocampus, LS and other brain regions that required much higher VP concentrations, may be of physiological significance and could be related to the behavioral effects of this neuropeptide.
415.5 EFFECTS OF CEREBROLYIN ON SYNAPTIC TRANSMISSION IN THE RAT HIPPOCAMPUS. L.M. Wolkowicz, S. Wang and A. Basiky. Department of Physiology and Clarke Inst. of Psychiatry, Univ. Toronto, Toronto, Ont, MSS 1A8, Canada.

Cerebrolysin (CB) is a brain tissue-derived mixture of polypeptides and amino acids that is widely used in treatment of dementias. To understand how CB exerts its therapeutic actions we applied it acutely to the rat hippocampal slices from 3-5 week old Wistar rats. CB was dissolved in saline and applied to slices for 5-10 minutes. CB inhibited the evoked field potential (F1) responses in the CA1 region of the hippocampus. The threshold of the effect was observed at 2μM/L and a maximal inhibition at 10-20μM/L. At 10μM/L FPs were inhibited by 59% (S.E. = 9.5, n = 11). In 5 out 11 experiments a long-lasting increase (28%, S.E. = 12.7) of FPs followed the inhibition. The effects were not associated with changes in the presynaptic fibre volley or paired pulse facilitation. Addition of 10μM bicineulid did not affect the CB action on FP, suggesting independence of GABA Receptors. Similar effects were observed in CA3 area of the hippocampus but not in the dentate gyrus where smaller inhibition (16%, S.E. = 1.5, n = 4) followed by 18.5% increase was noted. Given complex, albeit unknown composition of CB, it appears to have remarkably specific action on synaptic circuits in the hippocampus. Further studies are needed in order to elucidate the active ingredient(s) of CB. Supported by EBEWE, Austria.


Since somatostatin (SS) and gamma-aminobutyric acid (GABA) are co-localized in some neurons, the interactions between the actions of SS and GABA-ergic inhibitory postsynaptic potentials (IPSPs) in the CA1 pyramidal neurons of the pig hippocampal slices are investigated. SS (5S-14, 2 μM) induced a hyperpolarization of the CA1 neurons associated with a reduction in the input resistance of the CA1 (n=16). These effects were not blocked by picrotoxin (25 μM) (n=24) or phaclofen (1 μM) (n=16). Chelation of intracellular Ca2+ (Ca2+-EGTA) with BAPTA (5 μM) or inhibition of protein kinase C (PKC) with sphingosine (30 μM) (n=4) had no significant effects on the hyperpolarizing action of SS. The peptide suppressed the GABA Receptor-mediated fast IPSPs and the GABA Receptor-mediated slow IPSPs, but had no significant effect on the excitatory postsynaptic potentials (EPSPs) (n=16). SS-induced depression of IPSPs was not due to the hyperpolarization of the neurons. SS-induced hyperpolarization of the CA1 neurons was greatly reduced in the presence of baclofen (20 μM, n=6), an effect that was not due to the hyperpolarization by baclofen. The presence of QX-314 in the CA1 neurons, prevented the hyperpolarization of the neurons by SS and baclofen (n=12). QX-314 blocked the depressant effect of the peptide on the fast IPSP but not the depressant effects of baclofen on the IPSP and the fast IPSP (n=12).

These results indicate that SS depresses GABA-ergic IPSP without affecting the GABA receptors. The peptide suppresses the fast IPSP through a QX-314-sensitive postsynaptic mechanism. SS can modulate IPSPs if the peptide receptors and the postsynaptic GABA Receptors are coupled to the same channels or the peptide and the amino acide act through the same intercellular second messengers. (The authors are grateful to Astra Pharmaceuticals for the gift of QX-314.)


Human galanin is a 30 amino acid peptide which differs significantly from porcine, bovine and rodent galanin both in regard to sequence in the carboxy-terminus and extent of the peptide and its terminal amidation. Synthetic human galanin, prepared using a solid phase peptide synthesiser and purified to homogeneity using high pressure liquid chromatography, was administered to rats and humans in order to assess the biological activity of human galanin. Intravenous bolus administration of synthetic human galanin to conscious rats during glucose infusions produced blood glucose elevation and reduced circulating insulin, consistent with established effects of native galanin in the rat. Synthetic human galanin was infused for 90 min into healthy human volunteers at low and high levels (50 and 100 pmol/Kg/min, respectively) for 90 min or saline control (n=8). A 25g intravenous glucose bolus was administered 30 min into the galanin infusion. There was a small drop in systemic blood pressure and an increase in heart rate during the infusion. All subjects reported a metallic taste and hypersalivation. No significant effect of human galanin on plasma glycose, serum insulin (basal or stimulated) or C-peptide levels was observed relative to control. Plasma growth hormone levels rose even in the face of a glucose load in both low and high dose infusions. Thus, human galanin is an ineffective β-cell suppressant in humans, compared with rats, but significantly increases growth hormone secretion.

415.10 PASSIVE IMMUNIZATION TO GALANIN ALTERS BIOGENIC AMINE METABOLISM IN MALE RATS. S.M. Gabrieli and P.J. Krout. Dept. Psychiatry, Mount Sinai School of Medicine, New York, NY 10029 and Bronx VA Hospital, Bronx NY 10468.

Galanin is found in biogenic amine-containing neurons. In the present study, male rats were implanted with lateral ventricular cannulas. After surgical recovery, the absence of cerebral, prefrontal cortex or a specific anti-galanin serum were administered intraventricularly to freely-moving, conscious rats. Animals received two morning injections, 24 hours apart. Hypothalamic tissues were harvested 4 to 6 days after the second injection for analysis of biogenic amine and metabolite concentrations by HPLC with electrochemical detection. Concentrations of the serotonin metabolite, 5-hydroxy-indol-acetic acid (5HIAA) were significantly elevated 32% and 39% in anti-galanin and preimmune-treated rats in the medial basal and preoptic hypothalamus, respectively. Concentrations of 5HIAA were unchanged in the paraventricular and supraoptic hypothalami. Presumably, passive immunization sequesters released peptide. Thus, these data suggest that galanin plays an ongoing inhibitory influence on serotonergic neurotransmission, perhaps subsequent to increased serotonin release or monoamine oxidative activity. This indicates that a novel biological interaction exists between galanin and serotonin.
415.11

W-Acetyl-Aspartyl-Glutamate Potentiates Inward Currents in Rat Dorsal Lateral Geniculate Nucleus Neurons

By Scott R. A. Malaney* Department of Biology, Georgetown University, Washington, DC 20057.

W-acetyl-aspartyl-glutamate (WAG) is an endogenous dipeptide found throughout the brain and is proposed to have a neurotransmitter or neuromodulatory role in the central nervous system. In the visual system, high concentrations and a calcium-dependent release (Hoffett et al., Brain Res 538:86-94, 1991). Among the highest concentrations noted was that of the dorsal lateral geniculate nucleus (dLGN). Since retinogeniculate transmission is thought to be mediated by glutamate, and WAG has been reported to activate glutamate receptors, its effect was studied on dLGN neurones in the rat. 20 Hz pulses were used to whole cell patch clamp electrophysiology on 300 micron thick thalamic slices containing the dLGN. When WAG was applied via a pressure pipette (9 mM in ACSF, pH 7.3) on the recording site, cells clamped at the resting membrane potential (typically -60 to -75 mV) exhibited increases of inward current (as much as -1.14 pA) for varying periods of time before returning to baseline current values. Similar responses were elicited in glutamate (10 mM in ACSF, pH 7.3) applied in the same manner, suggesting that responses to WAG were mediated by glutamate receptors. These results suggest a role for WAG as a neurotransmitter in the dLGN.

416.1


We evaluated the role of endogenous neuropeptide Y (NPY) in the suppression of baroreceptor reflex (BRR) responses by locus coeruleus (LC), using adult, male Sprague-Dawley rats anesthetized with pentobarbital sodium (40 mg/kg, i.p.). Under an electrical stimulation condition (10 train of 1-ms rectangular pulses, at 10-20 μA and 10-20 Hz) that did not appreciably alter the basal systemic arterial pressure and heart rate, the LC significantly suppressed the BRR responses induced by an intravenous injection of phenylephrine (5 μg/kg), bilateral microinjection of NPY (4.65 pmol/20 μl), or an antiserum against NPY (120, 20 μl), into the nucleus tractus solitarii (NTS), the terminal site of baroreceptor afferents, elicited respectively a reduction in, and an enhancement of, the BRR response. Direct application of the NPY antiserum into the NTS also attenuated the suppressive effect of the LC on the BRR response. Treatments with normal rabbit serum, and heat-inactivated NPY or NPY antiserum, on the other hand, were ineffective. These results suggest that neurons in the brain that contain NPY may exert a tonic reduction in the BRR sensitivity. Furthermore, the endogenous NPY may participate in the modulation of the same reflex by the LC. Both these actions may possibly take place at the NTS.

416.2

EFFECT OF NEUROPEPTIDE-Y (NPY) ON BLOOD FLOW IN THE RAT TAIL AND FOOT. M. E. Heath and J. B. Thomas*, Navy Medical Research Institute, Bethesda, MD 20889.

The purpose of this study was to assess the effect of i.v. administered NPY on superficial cutaneous microcirculatory blood flow in the tail and foot (by laser doppler flowmetry), and on total blood flow in the tail (via venous occlusion plethysmography). Male 300 g Long-Evans rats, with jugular catheters, were anaesthetized, placed in a tubular plexiglass holder, and allowed to equilibrate for 25-30 min before experiments. NPY, norpinephrine (NE), [Leu]4, Pro36 NPY, NPY[13-36] or saline control was administered i.v. following 5 min of baseline data. Increasing doses of NPY, from 16 to 64 μg/kg, induced an immediate, marked, and long lasting decrease in superficial cutaneous microcirculatory blood flow in both foot (max=50%) and tail (max=25%). In contrast, total blood flow in the tail either showed no change or increased (max=25%). In addition, the volume of blood in the tail usually increased in response to NPY, thus suggesting relaxation of and reduced resistance in the larger tail vessels. NE and the specific Y1 receptor agonist, [Leu]4, Pro36 NPY, produced effects on superficial foot and tail blood flow and on total tail blood flow similar to that of NPY, although smaller in magnitude. NPY[13-36], a specific Y2 receptor agonist, did not appear to modulate blood flow in either the foot or tail. These findings suggest that NPY Y2 receptors do, and Y2 receptors do not, participate in the cutaneous microvascular blood flow response of NPY.

416.3


The regulatory peptides, VIP and NPY, are present in autonomic nerve fibers innervating the blood vessels. Exogenous VIP exerts vasoactive effects resulting in a decrease in blood pressure (BP), while NPY has vasocostricton effects resulting in increased BP. To study the role of endogenous VIP and NPY in the regulation of BP, we passively immunized rats against these peptides. Anti-VIP serum (AVS-1) and anti-NPY serum (ANS-1) were generated in rabbits against synthetic porcine VIP and NPY, respectively. VIP monoclonal antibody (VIP-1b) was supplied by J.C. Porter (Univ. of Texas). AVS-1 has a Bmax of 491nmol/Kg and Kd=0.83 nM for VIP and does not crossreact with any of 17 other peptides tested, including peptide histidine isoleucine. ANS-1 has a Bmax = 1.99 nmol/Kg and Kd=0.47 μM for NPY and does not crossreact significantly with rat peptide YY, rat pancreatic peptide or any of 8 other peptides tested. Arterial BP was continuously measured in anesthetized (ketamine & pentobarbitale) Sprague-Dawley rats (males, 220-250g) for 45 minutes beginning 5 min before treatments. BP was not changed after ANS-1 administration (1 ml, iv) when compared to BP in control rats (saline or normal rabbit serum). In contrast, when AVS-1 (1 ml, iv) was given, BP increased (144%, p<0.01). Heart rate was not changed. Similarly, VIP-1b (0.2 mg/kg) injected IP in a dose dependent manner (up to 138%, p<0.01). These results suggest that VIP may function as a tonic vasoconstrictor and be involved in the physiological regulation of BP in the rat, whereas the involvement of NPY in the maintenance of normal BP is not evident. (Supported by NSF DCB-900470).

416.4


Hypothalamic neuropeptide Y (NPY) has multiple behavioral and physiological functions and has been shown to produce both increases and decreases in neuronal activity. To determine the possible contributions of NPY receptor subtypes to these responses, the effects on neuronal activity of NPY and the presumed Y1 and Y2 agonists (Pro34)-NPY and C2-NPY were examined. Coronal slices of rat hypothalamus were maintained in vitro and extracellular single unit recordings have been obtained from paraventricular nucleus (PVN) neurons and 7 perifornical area (PFA) neurons. Cells in the PVN had a spontaneous firing rate of 3.2 spikes/sec; spontaneous firing rate for cells in the PPH was 7.8 spikes/sec. In the PVN, microtopical application of NPY and [Pro34] NPY produced a repeatable decrease (11/16 at 1.5 μM and 6/11 cells at 0.15 μM) on average, to 20% of spontaneous rates. The reduction lasted from 3 to 20 min. Similar responses to NPY were evident in the PPH. In contrast, C2-NPY (0.15 μM) application in the PVN predominantly increased firing rate an average of 79% in 59 cells. Excitation lasted an average of 4 min and most cells returned to their baseline firing rates. The present findings suggest that the NPY agonists have differential effects on neuronal discharge rate; perhaps via actions on different NPY receptors. Supported by NSF BNS 9008818 and NIH NS 24268.
415.6 NEUROPEPTIDE Y MODULATION OF MELATONIN SECRETION FROM RAT PINEALOCYTES. V. Simonneau, S. Bichet and P. Pévet. Lab. of Neurobiology of Rhythmic and Seasonal Functions, URA-CNRS 1332, 12, rue de l'Université, 67000 Strasbourg, France.

Pineal gland of mammals receives a noradrenergic input indirectly from the retina. The day/night rhythm of noradrenergine (NE) release, the major neurotransmitter of the day/night rhythm of melatonin synthesis and release with high nighttime values. NE added to cultures of rat pinealocytes increases melatonin secretion by pinealocytes previously stimulated with or not by the α2-adrenergic agonist isoprenaline (ISO). This study was performed on 2-day-old pinealocyte cultures of one month-old rats. NE alone induces a small stimulation of melatonin secretion. Together with ISO, NE displays a clear potentiation effect on melatonin secretion especially at low ISO concentrations. The most efficient concentration of NE in stimulating pineal activity is 10 nM.

In conclusion, this study demonstrates that NE stimulates melatonin secretion at a postomnic level in the rat pineal gland.


VIP acts on many cell types through mechanisms which are not fully understood. In the pineal gland, VIP increases CAMP accumulation and N-acetyltransferase (NAT) activity, the rate-controlling step in melatonin synthesis. It is known that stimulation of these parameters by noradrenergine (NE) is strongly dependent upon α1-adrenergic elevation of [Ca2+]i. Accordingly, in this investigation we determined whether [Ca2+]i, is also elevated by VIP, this was examined using computer assisted single cell analysis of pinealocytes in primary culture. VIP (1-100 nM) produces a rapid and sustained increase in [Ca2+]i, in more than 85 % of the cells, as does NE. This indicates that both agonists act on the same population of pinealocytes. The VIP-induced increase in [Ca2+]i is absent when [Ca2+]i is less than 1 J/m. In contrast, the NE response is reduced to a transient increase in these conditions, suggesting that VIP and NE may act in part through different mechanisms. The VIP-induced increase in [Ca2+]i is not abolished by classical voltage-gated Ca2+ channel inhibitors, such as nicardipine, D-600 and omega-conotoxin. The effect of VIP was mimicked by PPACK 1-28.

These studies indicate that VIP can elevate [Ca2+]i, and that it acts by opening plasma membrane channels which are distinct from classical voltage-gated channels.


VAGUS TUBE INTESTINAL PEPTIDE (VIP) IS A NEUROMODULATORT, GROWTH REGULATOR AND SECRETAGOGUE FOR NEUROGENAL SURVIVAL FACTORS (COXES AND BRENNER, 1989, NOL. NEUROBIOL. 3, 1-3).


416.10 ANTAGONISTIC PROPERTIES OF CORTICOP. 37 ON NEUROCHEMICAL EFFECTS OF HUMAN CALCITONIN GENE-RELATED PEPTIDE. D. Ménard, A.L. Drumheller, A. Pouinier, and F.B. Jolicoeur. Departments of Psychiatry and Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N1-RNS-Saml, Point Claire, Que, Canada.

Pre-administration of the C-terminal fragment hCGRP37 antagonizes some but not all neurobehavioural effects of hCGRP. In the present study, we investigated the effects of hCGRP administered alone or following pre-treatment with hCGRP on brain regional concentrations of noradrenergine (NE), dopamine (DA), DOCA, HVA, as well as somatostatin (S-ST) and 5-HIAA. Peptides were administered ICV in a dose of 20 µg and 20 min later, brains were removed and dissected for neurochemical analyses. No neurochemical changes were found in striatum or thalamus. However, hCGRP enhanced DA and increased HVA concentrations in the nucleus accumbens. Increased concentrations of all amines and metabolites were found in the globus pallidus, amygdala and frontal cortex. The hippocampus, except for HVA, all neurochemical parameters were elevated by hCGRP. In the hypothalamus, hCGRP significantly increased and decreased DOCA and HVA respectively without affecting other neurochemical parameters. In the substantia nigra, marked decreases in DA and HVA levels were the only significant changes found. Pre-treatment with hCGRP either significantly attenuated or completely abolished all neurochemical effects of hCGRP in the substantia nigra, amygdala, nucleus accumbens and globus pallidus. In the frontal cortex, except for the increase in NE, all neurochemical effects were blocked by hCGRP. In the hypothalamus, hCGRP induced increases in hippocampal DOCA and 5-HIAA concentrations were also not affected by the fragment. Finally, hCGRP37 did not alter any of the neurochemical actions of hCGRP in the hypothalamus. Together, these results reveal pervasive but heterogeneous neurochemical effects of hCGRP and point to the existence of pharmacologically distinct receptors mediating these effects. Supported by the Medical Research Council of Canada.
416.11  
PHARMACOLOGY OF THE EFFECTS OF BRADYKININ AND SEROTONIN ON THE RELEASE OF CGRP FROM THE RAT TRACHEA  
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Depolarization of peripheral terminals of C-fiber afferents in the rat trachea can induce a local release of CGRP. In a previous study, application of bradykinin (BK) into the tracheal perifusate induced a dose-dependent increase in CGRP release, while serotonin (5-HT, 10⁻⁶M) with no effect along, facilitated CGRP release by capsaicin (10⁻⁶M), but not by a BK antagonist: [Des-Arg⁹, Leu⁵]-BK. The facilitatory effect of 5-HT on CGRP release appeared mediated by activation of the 5-HT2 receptor and subsequent PG formation. The sensitization effect of 5-HT on the releasing function of primary afferent terminal is apparently mediated by a 5-HT3 receptor and prostaglandin synthesis.  
1. Society of Neuroscience Abstract 17: p395, 1991. (This work is supported by Tobacco Related Disease Program, University of California. X-Y. H.)

416.12  
ANATOMICAL DELIMITATION OF A NEW ANIMAL MODEL OF DEPRESSION USING CALCITONIN S. Soud and R. De Beersteine*, Laboratory of Pharmacology, CHU Côte de Nacre, 14032 Caen, France  
Calcitonin is a peripheral peptide hormone which has several functional, behavioral, and hormonal effects when injected into the rat cerebral ventricles or into specific brain sites: calcitonin decreases food intake, locomotor activity, intestinal motility, produces sleep disorders, increases cortisol secretion, and inhibits sex hormones secretion and TRH-induced TSH secretion. On the other hand, these calcitonin effects mimic the symptoms of the human depressive syndrome. Moreover, the anergic effect of calcitonin can be reversed by specific antidepressants. We therefore proposed that calcitonin is a mimic of the animals a new model of depression. Previous experiments have shown that many of the effects of calcitonin listed above are related to an effect on the periventricular nucleus of the hypothalamus. Calcitonin has also marked anergic and hyperthermic effects which are not common symptoms in depression. To clarify possible anatomical grounds for our model, we studied the brain sites involved in the anergic and hyperthermic effects of calcitonin.  
We tested the effects of intracerebral injections of salmon calcitonin (150g in 0.5μl) on heart rate, body temperature, and justification rate. The results show that the sites involved in calcitonin induced anergies and hyperthermias are the preoptic area, the dorsal-medial and posterior nucleus of the hypothalamus, the suprachiasmatic nucleus, the ventro-medial nucleus of the thalamus, and an area in the internal part of the zona incerta. The ventro-medial and the periventricular nucleus of the hypothalamus are not involved in calcitonin-induced multiple and hyperthermia.  
We conclude that calcitonin-induced anergies and hyperthermia can be excluded from our model of depression which may fit more specifically with a selective action of calcitonin on the periventricular nucleus of the hypothalamus.

416.13  
DYNORPHIN A (1-17) INDUCED DEPRESSION OF VENTRAL ROOT POTENTIALS IS MEDITATED THROUGH KAPPA OPIATE RECEPTORS. H. Rats and L. Isaac*  
Department of Pharmacology, University of Illinois College of Medicine at Chicago, IL 60680  
Intrathecal application of dynorphin A (1-17) (DYN) results in a dose-dependent hindlimb paralysis of 30-60 min duration which may be mediated through kappa opiate receptor interaction. Additionally, repeated injection of DYN at 2 hr intervals results in desensitization to paralysis. Finally, direct application of DYN to the spinal cord results in a dose-dependent depression of the ventral root potentials (VRP) of 30-60 min duration. To investigate this apparent parallelism we determined whether the VRP depression is kappa receptor mediated and whether it is subject to desensitization.  
Rats were surgically prepared, under urethane anesthesia, to record VRP monosynaptic and polysynaptic reflexes. The kappa opiate receptor antagonist nor-binaltorphimine (nor-BNI 20μm) was applied directly to the spinal cord 60 min prior to DYN application and in separate experiments DYN (10μm) was applied at 2 hr intervals to the spinal cord.  
Nor-BNI resulted in a partial shift of the dose-response curve for DYN-induced depression of the VRP. With nor-BNI the ED₅₀ was 8 μm and with saline it was 4 μm. Repeated application of DYN resulted in desensitization to depression of the VRP of about 50%.  
These data suggest that DYN-mediated depression of the VRP is mediated through the kappa opiate receptor. Because both DYN-induced paralysis and depression of the VRP are mediated through kappa receptor interaction they may be causally related. Although the mechanism of these effects remain to be investigated they both involve desensitization. Supported by NSF BNS-8916983 and NIH NS-30295.

416.14  
COMPARISON OF BEHAVIORAL EFFECTS OF DYNORPHIN A (1-17) WITH DYNORPHIN A (1-13) AFTER INTRATHecal INJECTION IN THE RAT. Z.X., Q.T. J. Marczynski* and L. Isaac Department of Pharmacology, Univ. of Illinois College of Medicine at Chicago, IL 60680  
Previously, we reported that dynorphin A (1-13) administered intracerebroventricularly to rats resulted in a reversible hindlimb paralysis and an irreversible inhibition of the tail-flick reflex. These effects follow a dose-response relationship with identical ED₅₀ values.  
In the present investigation, we report the influence of dynorphin A (1-17), the peptide configuration of the endogenous substance, on these same behaviors after i.t. injection in rats. Injection of this substance results in a reversible hindlimb paralysis and an irreversible inhibition of the tail-flick reflex. These effects follow a dose-response relationship with an ED₅₀ value for paralysis of 7 μmole and a value for inhibition of the tail-flick reflex of 18 μmole whereas the value for the (1-13) configuration is 10 μmole for both behaviors.  
With (1-13), paralysis and inhibition of the tail-flick reflex always occur together; on the other hand with (1-17), some animals paralyze while their tail-flick reflex remains intact suggesting that different mechanisms may be involved with the different peptide configurations.  
These data demonstrate a fundamental difference between dynorphin A (1-17) and dynorphin A (1-13). In conclusion, it appears that use of the (1-17) peptide to study the physiologic and pathophysiologic role of dynorphin in spinal cord function is preferred over the use of the (1-13) configuration.

416.15  
EVIDENCE FOR A DIRECT ACTION OF MET-ENKEPHALIN ON GABAergic TERMINALS IN ORGANOTYPIC SLICE CULTURES OF THE RAT HIPPOCAMPUS. J.C. Bechlin#, Institute of Neurophysiology, University of Copenhagen, DK-2200 N. Denmark  
Intracellular recordings from pyramidal cells in organotypic slice cultures of the hippocampus were performed with 1 M KCl containing extracellular solution. Spontaneous IPSPs were blocked by 20 μM NBQX, APV and synaptic transmitter release due to sodium-dependent action potential was blocked by TTX. Under these circumstances a tonic bombardment of miniature IPSPs were seen. The IPSPs were blocked by 20 μM bicuculline and thus were GABAergic.  
Application of 10-20 μM met-enkephalin reduced the frequency of spontaneous IPSPs by up to 50% and this effect was reversed upon wash: for 20 min.  
These observations point to the conclusion that pyramidal cells cultured in vitro and hippocampal are exposed to tonic GABAergic inhibition, which is not the result of propagating sodium-dependent action potentials, and that this tonic inhibition can be reduced by met-enkephalin through a direct action of met-enkephalin on the presynaptic terminals.

416.16  
EVIDENCE THAT PITUITARY ADENYLATE CYCLASE ACTIVATOR PEPTIDE (PACAP) IS A PRESYNAPTIC NEUROTRANSMITTER IN THE BOVINE ADRENAL MEDULLA. J.C. Wray, V. Hendrick and D. Martin  
The Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77030  
VIP has been shown to stimulate catecholamine (CA) synthesis in isolated bovine adrenal chromaffin cells. One of the peculiar aspects of this stimulation is the high concentration of peptide (μM) required, if VIP is mimicking the action of other peptides that act endogenously at lower concentrations. Accordingly, we tested the effect of PACAP, a VIP-like peptide, on the CA synthetic pathway in bovine adrenal chromaffin cells. These studies revealed a potent interaction of either PACAP-27 or PACAP-38. At a high concentration PACAP-38 stimulated 4.5-fold with a 1/2 max concentration of 20 nM. Tyrosine hydroxylase (TH), the rate limiting enzyme in CA synthesis, is phosphorylated selectively on serine 40 by both PACAP and VIP. At these points, TH mRNA synthesis and levels are elevated, followed by increased TH protein and activity. Morphological studies using perfusion-fixed, intact bovine adrenal medulla showed PACAP-27 immunoactive axons innervating BAC. Labeled axons and terminals were found throughout the medulla but were particularly dense near the adrenal cortex and around blood vessels. The labeling was unaffected by synthetic 1 μM VIP but completely abolished by synthetic PACAP-27. These results support the conclusion that PACAP is a presynaptic neurotransmitter in the bovine adrenal medulla and a promising candidate for the endogenous ligand of the "VIP receptor" described previously. Supported by USPHS grant NS311061 & BY06472.
**416.17**

PITUITARY ADENYLYL CYCLASE ACTIVATING POLYPEPTIDE (PACAP) REGULATION OF AIT-20/16V CORTICOTROPE CELL FUNCTION. K.M. Brasar, I.M. Koropka, C.A. Brandenburg and V.M. May. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405, and Department of Biological Psychiatry, Hines VA Medical Center, Hines, IL 60141.

The α-aminated hypothalamic peptides, pituitary adenylate cyclase activating polypeptide (PACAP) and PACAP27, share considerable amino acid homology with vasoactive intestinal peptide; however, the physiological roles of the PACAP peptides remain unclear. PACAP alters the functions of anterior pituitary gonadotropes, somatotropes and a small number of corticotropes. To investigate the roles of PACAP on corticotropes, we examined PACAP-mediated regulation of ACTH/endorphin secretion and membrane depolarization using AIT-20/16V mouse pituitary corticotrope cells. The basal secretory rate for AIT-20 cell ACTH was approximately 3% of cellular hormone content. Treatment of AIT-20 cells with 100 nM PACAP38 for 3 to 6 h stimulated hormone release approximately 1.5- to 1.8-fold. PACAP27 elicited a similar stimulated secretory response, suggesting that the PACAP peptide exerts functional responses on corticotropes cells by increasing action potentials; occasionally, these action potentials led to the initiation of sustained depolarizations (SD) (< 3 sec), followed by hyperpolarizations (< 1 sec). The input resistance significantly decreased during the plateau of the depolarizations. 100 nM PACAP38 increased the rate of SD approximately 2-fold, suggesting a possible correlation with increased hormone release. (Supported: NSF grant 9010444 to KMB, HD-27468 to VM and VA RAG 0001 to LMK)

**416.1**

THE PENETRATION OF DYNORPHIN-1-13 ACROSS THE BLOOD BRAIN BARRIER. J.L. Browning*, T.P. Turner, M.A. Widmayer and D.S. Baskin Dept. of Neurosurgery, VAMC Houston and Baylor College of Medicine, Houston, TX 77030.

The opioid peptide dynorphin 1-13 has been shown to increase survival and decrease neurologic deficit and infarct size in a feline model of focal cerebral ischemia (FCI). The ability of (Ile-His-Pro)1-13-dynorphin 1-13 (DYN) to cross the blood brain barrier (BBB) was studied in rats, cats and cats with FCI. The brain uptake index (BUI) was measured by a modified Oldendorf technique showed that the BUI of DYN was not significantly different from sucrose (vascular space reference) in the rat. Peptide inhibitors had no effect. In contrast, the BUI of DYN in the cat was significantly greater than that of sucrose in hippocampus, cortex and cerebellum. In addition, the BUI of DYN was even greater in cats with FCI, such that the BUI of DYN was significantly greater in hippocampus, cortex, brainstem and hypothalamus. These results suggest that DYN does cross the BBB of cats and may act centrally in ischemia to mediate its neuroprotective action.

**416.2**


We have previously demonstrated that β-endorphin (BEND) content is lateralized in the medial preoptic and caudal arcuate areas of the male rat brain, and that this lateralization is differentially modulated by left vs right hemispheric injury. To investigate the bases for these differences, we recently initiated a series of studies to further investigate the mechanism of this apparently lateralized control. In this first study we investigated lateralization of peptidic markers of the medial preoptic and arcuate; preproenkephalin (PENK) and neuropeptide Y (NPY) mRNA content. These studies were consistent with a demonstration that left caudal MBH BEND content is greater than right, and suggest greater forebrain opiomelanocortinergic activity originating in the left vs right MBH. We have previously demonstrated that MBH MAG mRNA content is correlated with MBH tyrosine hydroxylase (TH) mRNA content, suggesting interaction between MBH opiomelanocortinergic and dopaminergic activity (Neuropysiociology, in press). Consequently we are further investigating whether MBH TH mRNA is also lateralized and whether this lateralization is correlated with that of POMC mRNA.

**416.3**

TWO DIFFERENT OPIODYT CELL GROUPS IN LAMINA X OF RAT LUMBOSacRAL SPINAL CORD. A.P. Nicholas, Z. Xu and T. Hikata*. Department of Histology and Neurobiology, Karolinska Institute, Stockholm, 10401, Sweden.

Using a three-color immunofluorescence technique, the coexistence of enkephalin-like immunoreactivity (ENK-LI) with immunoreactivities for other peptides was examined in neurons of lamina X in the lumbosacral spinal cord of conscious, untreated rats. Briefly, animals were anesthetized, the lumbosacral laminae were removed, the dura mater excised and colchicine soaked gelatin pads were placed under the dorsal roots for 1-2 h and then removed. After a 48 h survival time, the animals were perfused with formaldehyde/glacial acid and the lumbosacral cord removed, frozen and cut (14 µm) on a cryostat. These sections were incubated overnight in 1:20 mouse anti-ENK, plus either a combination of 1:400 rabbit anti-cholecystokinin (CCK) and 1:200 guinea pig anti-gallamine (GAL), or a combination of 1:400 rabbit anti-neuronenrich (NT) and 1:400 goat anti-neuropeptide Y (NPY) antisera. Secondary antiserum (1:40; Jackson Immuno Research) consisted of a combination of AMCA (blue) conjugated donkey anti-mouse and FITC (green) conjugated donkey anti-rabbit, plus Lissamine Rhodamine (red) conjugated either to goat anti-guinea pig or to donkey anti-goat antibody. ENK was shown to coexist with CCK and GAL in adjacent large lamina X neurons from L1 to L5, while ENK coexisted with NPY in almost all smaller lamina X neurons from L5 to S2. In this caudal region, a few ENK/GAL/peptide cells were primarily a separate cell group. Thus, the present study showed that in lamina X of the lumbosacral spinal cord, there are at least two major populations of opioid neurons: a rostral group of large ENK neurons that are CCK and GAL positive and a caudal group of smaller ENK neurons that are primarily NPY (and sometimes PENK) positive. Previously, others have shown that CCK/GAL and ENK cells in lamina X of the lumbosacral cord project to higher brain centers and it has been suggested that they are involved with pain transmission.

**416.4**

ULTRASTRUCTURAL LOCALIZATION OF ENEKPHALIN AND GABA IN RAT SUBFORNICAL ORGAN. J.M. Pickett* and J. Chan Division of Neurobiology, Department of Neurology and Neuroscience, Cornell University Medical Center, New York, N.Y. (9021)

We examined the ultrastructural localization of Met₂-enkephalin (ME) and GABA in the rat subfornical organ (SFO). ME-like immunoperoxidase labeling was intensely localized to large dense core vesicles (DCVs) in axon terminals. These terminals formed symmetric synapses primarily on unlabeled dendrites and also were seen within perivascular spaces and in the parenchyma adjacent to fenestrated capillaries in the central and caudal portions of the SFO. GABA labeled soma, dendrites and terminals were numerous throughout the SFO. In dual labeled sections, immunoperoxidase labeling for ME was detected in a few terminals forming synapses with cells showing gold silver labeling for GABA, but most of the targets were without detectable immunoactivity. Other types of associations between ME and GABA included: colocalization within single axon terminals, convergence of separately labeled terminals on common targets, and appositions between separately labeled terminals. Single as well as dual labeled terminals formed symmetric synapses with unlabeled dendrites throughout the SFO. These results suggest that in rat SFO, GABA and opioid peptides may be released from separate, or sometimes the same, axon terminals to inhibit (symmetrical junctions) local neurons. Moreover, the release of one or both putative transmitters may be modulated by circulating hormones or axonal associations. The findings suggest that GABA may be involved in the inhibition of renal water, sodium and potassium secretion by oocytes at the level of the SFO (Grant support: MH00078, DA04600, HL18794).
**411.5** VENTRAL TEGMENTAL AREA NEURONS RECEIVE CONVERGENT GABAERGIC AND ENKEPHALINERGIC INPUT FROM THE SAME AND MORPHOLOGICALLY DISTINCT TERMINALS S.R. Sesack* and V.M. Pickel Dept. Behavioral Neuroscience, Univ. Pittsburgh, PA 15260 and Neurology & N. Med. Coll., NY 10036, NY 10021

We examined the ultrastructural basis for functional interactions between enkephalin (ENK) and γ-aminobutyric acid (GABA) in the rat ventral tegmental area (VTA), using dual immunoperoxidase-gold methods. Immunoreactive labeling for ENK or GABA, which were dually labeled for both substances. Immunoreactivity for ENK was intensely associated with dense-core vesicles localized along non-synaptic portions of the plasma membrane, while ENK immunoreactivity was associated with small clear vesicles, some of which aggregated at presynaptic specializations. GABA-labeled terminals, with or without ENK-immunoreactivity, formed synaptic symapses on unlabeled dendrites, while singly ENK-labeled terminals formed either symmetric or asymmetric synapses on unlabeled targets. Separately labeled GABA and ENK-immunoreactive terminals frequently converged on common dendrites, or were in direct apposition to one another. These results suggest that GABA and ENK (1) are colocalized in a subset of VTA terminals; (2) are differentially released from distinct vesicle populations, regardless of their co-distribution; and (3) may have opposing actions on common VTA neurons following their release from the same or different terminals, which would be consistent with the idea that GABA and opioid peptides may interact to modulate the activity of dopaminergic and non-dopaminergic VTA neurons. This work was supported by USPHS grants: NS08193, MH04392, MH00078, DA04600.


It is well established that, in the striatum, biosynthesis of the opioid peptide dynorphin (Dyn) increases following activation of D1 receptors and decreases following D1 receptor blockade. In the present experiments, we were interested in examining some of the molecular mechanisms which may mediate, or interact with, the dopaminergic regulation of Dyn biosynthesis in the striatum. We treated (twice daily for 7 days) rats with the dopaminergic agonist, apomorphine (APD; 5 mg/kg), caused a small but significant increase in Dyn-in the striatum; this increase was accompanied by increases in the immediate early gene products, c-fos and/or fra. Pretreatment of animals with a low dose (125 mg/kg) of MK-801, a non-competitive antagonist of NMDA receptors, blocked the increases in both Dyn and fosf/ra immunoreactivity. In a subsequent experiment, animals received unilateral 6-hydroxydopamine lesions of the substantia nigra (destroying the dopaminergic innervation of Dyn-containing neurons in the substantia nigra) and were injected with APD alone or together with MK-801. APO treatment alone caused a 4-fold increase in Dyn-in the striatum and an intense immunostaining of fosf/ra peptide expression in the lesioned striatum. However, in contrast to its effects in intact animals, MK-801 did not appear to have any effect on the APO-induced increase in fosf/ra expression in 6-OHDA lesioned rats although it did reverse the increase in Dyn. These data demonstrate that changes in fosf/ra expression are sometimes dissociated from Dyn and suggest that other factors in addition to fosf/ra may be involved in the nigrostrial regulation of Dyn expression.


Increases or decreases in transynaptic activity in the RA activate c-fos induction and Penk gene expression in a sequential manner. We investigated whether c-fos induction and Penk gene expression can be dissociated in RA. Increases in transynaptic activity by a neurosteroid (a metabolite of 5α-3β-SDH) converted a sequential induction of c-fos and proenkephalin (Penk) mRNA in RA. In hypophysectomized RA, the MTD-induced increase in Penk mRNA, but not c-fos mRNA was significantly reduced. In normal RA, ACTH treatment blocks the MTD-induction of c-fos mRNA and Penk protein without a significant effect on the MTD-Induction of Penk mRNA. Removal of transynaptic activity by adenral medullary explantation also produced a sequential induction of c-fos and Penk mRNA. However, in contrast, the induction of Penk mRNA was blocked by cycloheximide (2 mg/ml). Porcine c-fos mRNA was not a factor in the induction of Penk mRNA at 1 hr. In contrast, the drug-induced increase in Penk mRNA at 24 hr. These results demonstrate that the Penk gene is an independent gene and c-fos induction does not always result in Penk gene expression and Penk gene expression can occur in the absence of c-fos induction. (Supported by DA01457 and DT07243.)


We have previously reported that ventral tegmental area (VTA) microinjections of the selective opioid receptor agonist DAGO produce increases in extracellular nucleus accumbens (NAs) dopamine (DA), DOPAC, and HVA, assayed with high performance liquid chromatography with electrochemical detection. Interestingly, VTA microinjections of the selective μ-antagonist CTOP also produce increases in NAs DA and metabolites. The dose-dependent changes in NAs DA and metabolites was evaluated, and the μ-receptor selectivity of the response to CTOP (a substitution of the COOH-terminal amino acid) was compared with the response to a structurally dissimilar μ-antagonist, flunoxadrenaline (B-FNA: a furamate methyl ester derivative of naloxone). VTA microinjections of CTOP in the dose range 0.03 - 3.00 nmol/inject produced dose-dependent increases in NAs DA and metabolites. VTA 8 - FNA (1.0 μmol) produced increases in NAs DA and metabolites which were statistically similar to the increments produced by an equimolar microinjection of CTOP. One possibility is that these effects of CTOP and B-FNA may be mediated through actions at μ receptors located on GABAergic afferents to the VTA. Release of these afferents from opioid-inhibition could result in increased GABA-mediated inhibition of GABAergic interneurons intrinsic to the VTA. This increased inhibition of GABAergic interneurons would then produce a disturbance of mesolimbic dopaminergic neurons, resulting in the observed increases in NAs DA and metabolite concentrations. Therefore, under physiological conditions, GABAergic afferent to the VTA and GABAergic interneurons within the VTA may interact in a complex manner to modulate mesolimbic dopaminergic activity.

**411.8** REGULATION OF C-FOS and 28 K CALIBINDIN mRNA IN RAT CEREBELLM IN RESPONSE TO ACUTE AND CHRONIC MORPHINE. P.S. Tsujimula and R.D. Howell*, Department of Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

The cellular and biochemical adaptations which underlie the addictive state are poorly understood. Since it is likely that changes in gene expression accompany the development of addiction, we are examining changes in the expression of candidate genes. In this study, the effect of acute and chronic morphine administration on expression of the proto-oncogene, c-fos, and the calcium-binding protein, calbindin, was examined in rat cerebellum and rat brain (minus the cerebellum). Adult male Sprague-Dawley rats were injected with morphine (10 mg/kg) daily for 15 days. Rats were sacrificed 45 min after the last injection, the cerebellum and remaining brain minus the cerebellum were removed, and total RNA was extracted using the acid guanidium thiocyanate/phenol chloroform method. RNA levels were quantified by Northern blot analysis. In addition, other rats received a single injection of morphine (10 mg/kg) and were sacrificed after 45 min, 4 hr, or 24 hr later. The effect of naloxone-precipitated withdrawal on gene expression in morphine-addicted rats was also analyzed 45 min after naloxone (1 mg/kg ip). Levels of c-fos mRNA were increased 2.5-fold in cerebellum and brain 45 min after a single dose of morphine compared to saline-injected controls. Calbindin mRNA levels in cerebellum were decreased to 30%-40% control at 45 min and 4 hr after a single morphine injection. Tolerance developed to these effects in that levels of c-fos and calbindin were not altered 45 min after morphine injection in morphine-addicted rats. Unlike the cerebellum, calbindin mRNA was increased 3.5-fold compared to controls 45 min after morphine injection in chronically injected animals in the remainder of the brain. Naloxone-precipitated withdrawal caused a 30% decrease in c-fos levels in the brain minus the cerebellum. Differential effects on c-fos and calbindin gene expression following either acute or chronic morphine administration may be important aspects of the adaptation of the nervous system to morphine. Supported by DA 05819.

**411.10** MU-OPIOID ENHANCEMENT OF NMDA AND AMPA RESPONSES IN HORIZONTAL Versus CORONAL SECTIONS OF RAT LOCUS COERULEUS. R. Miczek and J.T. Williams, The Vollum Institute, Oregon Health Sciences University, Portland, Oregon, 97201.

The μ-opioid receptor agonist DAGO was recently reported to selectively enhance the NMDA component of the glutamate response in spinal trigeminal neurons via activation of protein kinase C (Huang, 1991). We have investigated this modulation by μ-agonists in rat locus coeruleus (LC) neurons where μ-agonists elicit inhibitory effects through augmentation of a K+ conductance. Intracellular voltage clamp recordings were performed on LC neurons (holding potential of -70 mV) in horizontal or coronal brain slices. All drugs were applied by superfusion. Following pretreatment with diazepam (1 μM) and picotinin (100 μM), the responses to NMDA (100 μM) were increased by DAGO (1 μM) by a factor of 1.9 (167 - 12 versus -370 ± 30 pA). This enhancement was observed in horizontal sections of the LC (n=10) but not in coronal sections (n=6; factor of 1.1) nor in horizontal sections treated with 2 mM barium (n=3; factor of 1.1). The specificity of the DAGO-modulated enhancement was tested following application of AMPA (100-300 mM). The DAGO-enhanced current (factor of 1.2 ± 0.3) was potentiated by a factor of 2.3 by DAGO (275 ± 68 pA) in horizontal sections (n=4) but not in coronal sections of the LC (n=4; factor of 1.6). Current-voltage relationships from horizontal and coronal sections indicate a convergence of the AMPA and AMPA/DAGO regression lines. A lack of voltage clamp control of the dendritic arborization may explain the enhanced responses to DAGO and the following DAGO application. (Supported by FRSQ and NIH DA04523.)
411.13 CHRONIC MORPHINE INDUCES A PERMANENT INCREASE IN RAT STRIATAL CALBINDIN D28K IMMUNOREACTIVITY VIA AN NMDA RECEPTOR-DEPENDENT MECHANISM. M.M. Garcia* and R.E. Harlan, Dept. of Anatomy, Tulane University Medical School, New Orleans, LA 70112.

Calbindin D28K (CBD) is an intracellular calcium-binding protein which acts as a Ca++ buffer, thus protecting cells from the damaging effects of high intracellular Ca++. As chronic morphine (M) has been reported to cause increased Ca++ levels in striatal synaptosomes, we studied its effect on CD immunoreactivity (IR) in rat brain, using immunocytochemistry. In the initial series of experiments, male rats were made tolerant and dependent by sc implantation of 75 mg M pellets (1/day for 5 days); controls were implanted with vehicle pellets. Using a monoclonal antibody to CD, we found CD IR in control brains in the matrix compartment of striatum but not in patches. In the brains of M-treated rats, there was a dramatic increase in CD IR in matrix, with the appearance of intense CD IR in patches. When M treatment was discontinued, the increased CD IR in patches persisted through day 14 post-pellet. Because striatal patches receive glutamatergic (GLU) input from cortex, we studied the interactions between M and GLU in a second series of experiments using the NMDA antagonist, MK-801. Rats were injected daily for 5 days with saline/GLU, 5/M (10 mg/kg), MK (2 mg/kg)/GLU, or MK/GLU. Levels and patterns of CD IR in brains of S/30% MK/5 rats were as found in controls from the previous study, while the S/30% MK rats resembled the pattern seen in the M-treated brains. CD IR in the MK/GLU brains, however, resembled control patterns, with CD IR absent from patches. We suggest that chronic morphine increases glutamatergic transmission in striatum, increasing Ca++ and CD levels. These findings are consistent with reports that MK-801 inhibits tolerance to morphine, and provide a possible mechanism for this inhibition. (Supported by the National Institute on Drug Abuse)

411.14 MORPHINE REGULATES EXPRESSION OF JUN FAMILY MEMBERS IN RAT BRAIN. R.E. Harlan, O. Prakash* and M.M. Garcia, Department of Anatomy, Tulane Medical School (REH, MMG) and Division of Research, Ochsner Medical Foundation (OFC), New Orleans, LA 70112.

Members of the Jun family of immediate-early genes dimerize with c-fos or fos-related antigens, to form a protein complex which binds to the AP-1 promoter sequence, activating or inhibiting expression of a variety of genes. We have used immunocytochemistry with antibodies to c-jun (Oncozyme Science AB-1 and AB-2) and to Jun-B and Jun-D (gift of R. Bradski) to study the cellular distribution of these proteins, and their regulation by morphine. In untreated rat brains, the four antibodies reveal four distinct distributions, suggesting that they may recognize four Jun family members. The distributions of cells recognized by the c-jun AB-1, Jun-B and Jun-D antibodies are very similar to recent reports on distributions of cells expressing the c-jun, Jun-B and Jun-D genes. Since the distribution revealed by the c-jun AB-2 antibody is strikingly different, with immunostaining of neurons only in the nucleus accumbens, striatum, olfactory tubercle and central nucleus of the amygdala, this antibody may recognize a Jun-related antigen. In the striatum, the AB-2 antibody recognizes neurons in the matrix, leaving patches unstained. Chronic morphine treatment (one 75 mg morphine pellet, sc, daily for 5 days) greatly increased the number of neurons stained by the AB-2 antibody, especially in the patch compartment. Acute 30 mg/kg or chronic treatment with morphine greatly increased the number of neurons stained by the Jun-B antibody in the nucleus accumbens, striatum and frontal cortex. These results extend previous work demonstrating morphine-regulated induction of immediate-early gene expression (c-fos) in the striatum and further suggest that the activated mu-opiate receptor may be coupled to stimulatory as well as inhibitory cellular events. Supported by DA-06194, NS-24148 (REH) and DA-05411 (MMG).

411.15 HYPERTHERMIA INDUCED BY INTRAPEPTIC MICRODIALYSIS OF A SELECTIVE μ OPIOID RECEPTOR AGONIST IN RAT. L. Xu*, E.B. Geller and M.W. Adams, Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

Previous studies from this and other laboratories have shown that the hyperthermic response of the rat to opioids are mediated by μ receptors. To investigate the role of the opioiic area (POA) in the hyperthermia, the present study used the selective μ receptor agonist, PE-301 (7 mg/kg sc). The temperature change was measured in the POA (superior hypothalamic area) and the thermoregulatory areas (anterior hypothalamus). The rats were exposed to continuous subcutaneous infusions of PE-301 for 6 hr. The results showed that PE-301 produced a significant increase in body temperature.
418.1 ELECTROPHYSIOLOGIC EVIDENCE THAT VENTRAL PALLIADAL (VP) DOPAMINE MODULATES VP RESPONSES TO AMYGDALA STIMULATION.
The VP is a dopaminergic brain region that also receives amygdaloid (AMN) efferents. Receptor subtypes involved in dopamine (DA)-mediated VP responses and the effects of DA on AMN inputs to VP are unknown. Thus, the present study determined whether VP responses to AMN stimulation could be modulated at the level of the VP by exogenous DA (microlithophoretic application) and/or endogenous DA (stimulation of midbrain dopaminergic regions; mDA).

Single, spontaneously active VP neurons, recorded in vivo in male Sprague-Dawley rats and anaesthetized with chloral hydrate, were assessed for convergent responses to AMN and mDA single pulse stimulation. Most VP neurons tested responded to both. Seventy percent of the short latency inhibitory VP responses to activation of the mDA were attenuated by microlithophoretically applied SCH23390 (61.6% vs. 30.5%), and with a greater magnitude (61.7% vs. 30.5%). AMN-evoked short and long latency inhibitory effects of VP neurons were attenuated by DA within the VP (84% and 80%, respectively), and by prior mDA stimulation (10 pulse train; 85% and 78%). The results suggest a monosynaptic inhibitory influence on VP neurons by mDA that is often mediated through DA receptor activation. DA within the VP and mDA activation produce a similar modulatory influence on AMN efferents to the VP. Thus, the VP may be an integrative site of limbic (AMN) and motor (mDA) systems. Work supported by MH45180.

418.3 MOTRIC ANALYSIS OF DOPAMINE RECEPTOR SUBTYPE ACTIVATION WITHIN THE VENTRAL PALLIADAL AND DORSAL GLOBUS PALLIDUS.
T. C. Nagler* and F. Rehahn. Department of Pharmacology, Loyola University Chicago, Stritch School of Medicine, Maywood, IL, 60153.

Dopamine (DA) projections ascending to forebrain striatal regions are known to mediate a complex repertoire of motor-related behaviors. Recent work demonstrates that pallidal regions also are dopaminergic, responding to both D1 and D2 DA receptor activation. Thus, the present study investigated the effects of DA-mediated motor functions, and whether the contribution of the dorsal globus pallidus (DOP) differs from that of the more medial ventral pallidus (VLP), were examined.

Under pentobarbital anesthesia, male Sprague-Dawley rats were implanted bilaterally with guide cannulae to allow i.c.v. microinjection of treatments into the DOP and VLP. Experimentation began 1 week after surgery and various motor effects were quantified for 1 hr following i.c.v. treatments. Intrapallidal DA (0.01 to 100 ng/5 µl) induced a dose-related increase in locomotion and rearing/wall climbing. Quinpirole (QUIN, D2 agonist) elevated these behaviors only when injected into the DOP. In the VLP, such effects were mimicked by SKF82958 (SKF, a full D1 agonist). SKF also produced robust "mouthing movements" that remained intact in rats with complete lesions of endogenous DA (in contrast to the other behaviors). Thus, DOP and VLP regions contribute a distinct profile to dopaminergic modulation of motor function. Since the magnitude of the locomotor and rearing/wall climbing measures was less than that observed in these animals with 1 mg/kg ip amphetamine, pallidal structures serve as constituents of an engaged system for these behaviors. Whereas, mouthing movements resulting from VP D1 receptor activation, without the involvement of endogenous DA (and thus, D2 receptor) induced a response that was greater than any other treatment assessed, suggesting that VP D1 receptors play a critical role in this behavior. Supported by MH45180.

418.5 EXCITATION OF RAT CAUDATE NEURONS BY INDIRECT AND DIRECT ACTING DOPAMINE AGONISTS.
The effects of dopaminergic (DA) drugs were evaluated for effects on neuronal firing rates of DA neurons in the substantia nigra pars compacta (SNPC) and the caudate nucleus (CN), the major projection area for SNPC DA neurons, in choroidal hydrate anesthetized rats. Using dye-filled glass microelectrodes, DA neurons in substantia nigra pars compacta (SNPC), Bursten et al., JPTT 155:560, 1973) and caudate neurons were identified by classical electrophysiological/neuroanatomical criteria. Cells were located in central caudate and were selected for spontaneous activity and large positive-negative waveforms (Type II, Life Sci 25:419, 1979). Intravenous injections of the indirect DA agonist, amphetamine (AMPH, 1-3 mg/kg), excited spontaneously active CN neurons and inhibited SNPC neurons by a HAL-sensitive mechanism. Similarly, quinpirole (QUIN, 0.3-3 mg/kg), a D2 agonist, and apomorphine (3 mg/kg), a D1/D2 agonist, excited spontaneously active CN neurons by HAL-sensitive mechanisms. However, much lower doses of apomorphine were effective in inhibiting SNPC neurons. SKF38395, a D1 agonist, did not alter CN firing rates or responses to QUIN. The data is consistent with the view that, while apomorphine inhibited CN and apomorphine inhibited SNPC neurons by direct autoreceptor activation.

418.6 ELECTROPHYSIOLOGICAL STUDIES OF (-)-STEHODILENE ON SNC AND VTA DA NEURONS.

(-)-Stepholidine is a novel DA receptor agonist, but it possesses agonistic action on rotational behavior in the 6-OHDA-lesioned rats. It is presumed that D2 receptors in the SNR are involved in this agonistic action. The present work attempts to elucidate whether SFD exhibits the agonistic or antagonist action on extrastriatal firing recorded from SNR and VTA DA neurons in the anesthesized, the reserpinized, and the 6-OHDA-lesioned rats.

It has been shown that SFD had antagonistic effect only to D2 receptors in the SNR and VTA on anesthetized rats. However SFD was an antagonist on both D1 and D2 receptors in the reserpinized rats. Interestingly, SFD had the characteristic of depolarization inactivation in VTA, not in SNR, of the anesthetized rats. This action is the characteristic of atypical neuroleptic. Thus SFD would exist the potential possibility of atypical neuroleptic. Furthermore, when firing was recorded from the SNR in the 6-OHDA-lesioned rats, SFD showed the D1 agonistic action. This result would be compatible with the rotational behavior.
418.7 DIFFERENTIAL INTERACTION OF DOPAMINERGIC D-1 AND D-2 RECEPTORS WITH GLUTAMAMERIC AND CHOLINERGIC TRANSMISSION IN THE 6-HYDROXYDOPAMINE MODEL OF PARKINSON.
M. Morelli, S. Fenu, A. Coroglio, A. Pini, and G. Di Chiara, Dpt. of Toxicology, University of Cagliari, Cagliari, Italy.

The interaction of dopaminergic D-1 and D-2 agonists with antagonists of N-methyl-D-aspartate (NMDA) or muscarinic receptors, was examined after a unilateral 6-hydroxydopamine lesion of the dopaminergic nigrostriatal system. Blockade of NMDA receptors by MK-801 increased the contralateral rotational behavior induced by the D-1 agonist SKF 38393 and inhibited the rotation induced by the D-2 agonist LY 171558. Blockade of muscarinic receptors by scopolamine, like MK-801, potentiated D-1 mediated contralateral rotation but did not influence D-2 mediated rotation. D-1 dependent activation of the proto-oncogene c-fos in the lesioned caudate-putamen (CPu) was increased after both treatments. 2DPC utilization studies evidenced an increased metabolic activity in the entopeduncular nucleus and substantia nigra reticulata after these treatments, indicating that the behavior observed is associated with specific biochemical modifications in the CPu, reflected by functional modifications in striatal afferent areas. The results suggest that administration of NMDA or muscarinic receptor antagonists might improve the therapeutic efficacy of D-1 agonists in the treatment of Parkinson’s disease.


6-OH dopamine (6-OHDA) is known to selectively destroy nigrostriatal pathways in the brain. In this study, we have investigated the effects of 6-OHDA lesions on G protein levels in the rat striatum. Male Sprague Dawley rats were treated with unilateral lesions of 6-OHDA and striata were removed from both hemispheres 1, 4, 8, or 16 days post-lesion. Quantitative immunoblotting of western blots, using specific antisera, was used to assess relative levels of Goα, Gβγ, and Gαq subunits. Both Gαq and Gβγ levels were found to be depressed in striata ipsilateral to the lesion relative to non-lesioned striata, and this effect was most pronounced by day 16. Although both Gαq and Gβγ levels showed similar patterns of alteration, Gαq levels consistently showed the greatest difference for each day post-lesion, suggesting that alterations in Gαq levels may follow changes in Gαq activity. Gαq levels showed no significant alterations in lesioned relative to unlesioned striata at any day post-lesion. Given that the molecular basis of many human neurodegenerative diseases are not at present well established, we have begun attempts to relate these results to healthy individuals and to the alterations in human neurodegenerative conditions. This work was supported by grants from the Parkinson’s Foundation of Canada and NIH.

Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

Indirect dopaminergic agonists, such as amphetamine, typically increase motor-related, but suppress nonmotor-related neurons in rat striatum (Har et al., Brain Res. 489:365, 1989). In an initial attempt to assess the mechanisms by which dopamine regulates striatal activity, we monitored the effects of quinpirole (171558), a dopamine agonist that has a high affinity for D2 and, especially, D3 receptors, and SKF-38393, a D1 agonist, on single unit activity in the striatum of awake, behaving, male rats. Like amphetamine, quinpirole (1-5.0 mg/kg) alone, or administered 30 minutes after the D1 agonist SKF-38393, inhibited nonmotor-related neurons in the striatum. Unlike amphetamine, quinpirole also inhibited motor-related neurons located in the lateral and central striatum. In medial striatum, however, neurons were particularly sensitive to the animal's state of arousal. After quinpirole, these cells showed complex changes in firing rate that may, in part, reflect behavioral activation induced by the drug. In contrast, SKF-38393 (5mg/kg sc) alone had no effect on the firing rate of striatal neurons. Nor did its effects differ from control when administered 30 minutes after quinpirole. Collectively, these results suggest an important role for D2 and/or D3 receptors in regulation of striatal activity. Supported by USPHS Grant DA 02451.

418.10 SOME STUDIES ON DA D1 & D2 ANTAGONIST-INDUCED CATALEPSY IN RATS. D.M. Jackson1, J.A. Watson2, A. Bengtsson1, and M. Lindner.

Arcus Astra AB! and Astra Parn Control AB/2, Södertälje 151 54, Sweden.

Catalepsy (C) is produced in rats by either DA depletion or by blockade of DA D1 or D2 receptors with antagonists. While C is a useful & predictive model, the measure itself is sensitive to environmental manipulations. In this test study we investigated the method of testing on SKF38393 (SKF) and raclopride (R)-induced C. C was determined on a steel grid at an angle of 60° +2° & the time each rat spent before moving 1 of its 4 paws determined. 3 experiments are described. 1) 2 methods were used to measure C after agonist injection. First, the C time for each rat was determined 0.5, 1, 2, 4, 8, and 24 h after injection. Secondly, the C time was measured only once on each rat at 0.25, 1, 2, 4, 8, and 24 h after injection. With the first technique, both SKF & R produced dose dependent C that peaked 2 to 8 h after R & 0.5 to 1 h after SKF, peak time depending on dose. With the second technique, C was measured only once on each rat at 8 h after injection. In contrast, R produced weak C when each rat was tested once. 2) Rats were injected with R (60 μmol/kg). Half were injected 10 min later with saline & the remaining remained untreated. All rats were then tested repeatedly beginning 0.5 h after saline injection for 24 h. The time course curve for C was altered by the saline injection. 3) Rats were given R (60 μmol/kg) & half handled each 5 min, & half not handled, before determining C 0.5 h through 24 h after injection. Less C was evident in the handled group. It is clear that D1 & D2 induced C depends upon markedly different mechanisms, as proposed earlier. Some form of conditioning, which is extremely sensitive to environmental influences, seems to play a major role in D2, but not D1, induced C.


Rats deprived of REM sleep 96 hours using a water tank procedure; group L/S resided on larger (control) pedestals for 72 hrs, then on small pedestals for 24 hrs; control group remained on large pedestal for 96 hrs; and group CC remained in the home cage. The striata were analyzed for dopamine D1 and D2 receptor density and affinity using [3H]SCH23390 and [3H]YM-09151-2 as ligands, respectively. Group REMD showed increased in Bmax for D1 and D2, and increased Kv for D2, compared to group TC. Group L/S had increases in Bmax and Kv for D1 and D2, compared to group CC. Group TC showed decreased Bmax for D1 and D2 and decreased Kv for D2, compared to group CC. Thus, stress caused different receptor types, whereas REMD had an opposite effect. These data suggest that differences in D1 and D2 receptor binding may be used to distinguish the effects of stress from the specific effects of REMD. (Supported by Dept. of Army)
418.13
COMPARATIVE STUDIES OF F-18 LABELED BENZAMIDES AND [F-18]MNETHYLPSIPERONE: R1 Mace, 1 G. Greenberg, 1 P A. Pavlik, 1 D. Evens, 1 B. Ehrenkranz, 2 R. Landeck, 2 CD Unsworth, 2 K. Jones, 2 S. Childers, 2 RB Molinoff, 1 and M. Stylik. 1 1Univ. of Pennsylvania and 2Bowman Gray School of Medicine.

A series of imaging studies using PET were carried out on a baboon comparing the uptake and retention of 2,3-dimethoxy-N-(p-fluorophenyl)piripendol-4-yl benzamide (MIBP) and 2,3-dimethoxy-N-0-(p-fluorophenyl)9-aracyclohexyl-3,3,1 nonas-8-yl benzamide (MABN) with that of N-methylpsiperonine (NMSP). All three compounds displayed a high accumulation in the basal ganglia (BG) with NMSP exhibiting the lowest rate of washout from the cerebellum (Cb). MIBP reached a 40% BG uptake at 40 min post-injection. Washout of MIBP after maximal accumulation occurred with a half-life of 140 min. MABN and NMSP did not washout from the BG and a linear increase in the BG/Cb ratio was seen over the three hour data acquisition period. The rapid washout of MABN from the Cb resulted in a BG/Cb ratio twice that of NMSP. A series of in vivo binding studies were conducted in order to determine the selectivity of each analog for D2 vs 5-HT2 and D2 receptors. The radioligands and tissue sources used were: D2, [3H]NC-298, rat striatum, 5-HT2, [3H]II-LSD, P11 cells, and [3H]Rauwolscine, rat cortex. The results of the in vivo study indicate the MIBP and MABN possess a higher 5-HT2/D2 ratio than NMSP. All three analogs possess a low affinity for D2 receptors. The results of these studies suggest that MIBP and MABN may be superior to NMSP for PET studies of D2 receptors.

<table>
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<tr>
<th>Compound</th>
<th>D2</th>
<th>5-HT2</th>
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<td>3000</td>
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418.15
REVERSED RELATIVE EFFICIENCIES OF QUINPIROLE (QUIN) AND (+3-PPP AT DOPAMINE (DA), D. RECEPTORS (D, R) IN STRIATUM AND ANTERIOR PITUITARY (AP); E. Meier, 1 J. Pizz, 2 J. Diamond, 1 and K. Bohmaker, Dept. Psychiatry, NYU Medical Center, New York, NY 10016.

Previous studies have shown that D3R on striatal DA nerve terminals and AP luteocorticol mediating, respectively, inhibition of transmitter synthesis and prolactin (PRL) release, both display a similar large receptor reserve (RR) for full agonists. Both effects involve receptor coupling to pertussis toxin (PTX)- sensitive G protein(s); consequently, the efficiency of receptor coupling (i.e., RR), is likely to be related to the ability of agonists to induce ternary complex formation. Treatments which reduce the amount of receptor or G protein are therefore expected to reduce the RR. Treatment of AP cultures with 17-estradiol (EST; 10, 3 days), recently shown to reduce PTX-sensitive G protein levels in AP, shifted the dose-response curve (DRC) for DA-4-propylnorapomorphine (NPA) to the right (14-fold; receptor inactivation is PTX-sensitive; 1 μM, 30 min) showed that EST treatment abolished the RR for NPA. Surprisingly, EST had nearly identical effects on the DRCs for QUIN and (+3-PPP (4.3-fold shift), although differential effects were expected since (+3-PPP has been found to be a much weaker agonist than QUIN in striatum. Treatment of cultures with PTX (10 ng/ml, 24 hr) produced shifts in the DRCs for NPA, QUIN and (+3-PPP which did not correlate with their previously determined relative efficacies; the PTX DRCs were also significantly shallower (P < 0.001). Receptor inactivation experiments revealed that maximal response in AP for QUIN and (+3-PPP required 25 and 9.6% receptor occupancy, but 6.2 and 3.0%, respectively, in striatum. "Promiscuous" coupling of D3R to different G proteins in the two tissues may underlie the reversal of relative efficacy, as recently predicted (Konakini and Morgan, Mol. Pharmacol. 1989). Supported by NS 23618.

418.16
LOCALIZATION AND BINDING ANALYSIS OF [3H]BTPC: A DOPAMINE UPTAKE SITE ANTAGONIST. M.E. Hunt1, P. Filloux, 2 H. Marang, C. Johnson, and J.R. Wamsley1. 1Research Institute, Fargo, ND 58103 and Western Institute of Neuropsychiatry, Salt Lake City, UT 84106.

The phenylpiperidine analog, [3H]-BTPC: (4-(3-benzoylphenyl) piperidine) has recently been made available in tritiated form ([3H]BTPC) for use as a radioligand. Specific binding of [3H]BTPC in striatal tissue was maximized at a pH ranging from 6.8 to 7.0 and a NaCl concentration between 145 and 220 mM. Nonlinear regression analysis of association and dissociation experiments yielded a Kd = 0.008 min^-1 and a Ka = 0.023 min^-1. The calculated Kd is equal to 30.22 min^-1 which is in agreement with the k_d derived from nonlinear regression analysis of the dissociation isotherm for a single high site model. However, two site modeling of the saturation isotherms proved to be significantly better than one site model and revealed high and low affinity binding sites with k_d’s of 1.3min and 79.7min respectively. Autoradiographic localization showed high binding in the caudate putamen, nucleus accumbens, and olfactory tubercule.
419.1

**ELECTROPHYSIOLOGICAL ACTIVITY MODULATES THE LEVEL OF ENDOGENOUS ADENOSINE IN THE HIPPOCAMPAL SLICE.** \*J.B. Mitchell* and T.W. Dunwiddie. University of Colorado Health Sciences Ctr. and VA Medical Ctr., Denver, CO 80262

Adenosine is a potent inhibitory neurotransmitter within the CNS. An increase in adenosine levels accompanies hypoxia, ischemia and seizures, but it is not known if elevating adenosine has a role in non-electrophysiologic function. We have found evidence for the activity-dependent release of adenosine from in vitro hippocampal slices maintained under physiological condition. Bipolar stimulating electrodes were positioned in the Schaffer collateral-commissural fiber layers of areas CA1 and CA3, and test fEPSPs were recorded from a single recording electrode positioned in the stratum radiatum of CA1. A train of conditioning pulses was applied to one stimulating electrode, and then a response evoked by applying a test pulse to the second stimulator. A train of 6 conditioning pulses (100 Hz) beginning 250 ms prior to the test pulse decreased the amplitude of the test fEPSP to 78 ± 1.9 percent of control. Antidromic firing of the CA1 pyramidal cells, evoked by an electrode positioned in the alveus, decreased the amplitude of the test fEPSP by 81 ± 2.6 percent of control. The decrease in fEPSP amplitude induced by either conditioning stimulus could be prevented by superfusion with an adenosine antagonist. When 2 conditioning pulses were used, the decrease in fEPSP amplitude lasted 500 ms and the maximum decrease occurred at 250 ms. Superfusion with the adenosine uptake inhibitor, dipyridamole, (50 μM) lengthened the duration of the decrease. Threogenesis adenosine levels can be increased in response to activation, and that this adenosine can then decrease excitatory neurotransmission in the hippocampus. 

Supported by NS29173, VA Medical Research Services, MRC of Canada.

419.2

**NMDA- AND NON-NMDA-EVOKED ADENOSINE RELEASE: DISTINCT PURINERGIC SOURCES AND MECHANISMS OF RELEASE.** \*C.G. Craig* and T.D. White. Dept. of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

Activation of excitatory amino acid receptors releases the inhibitory neurotransmitter adenosine from superfused rat cortical slices. Here we investigated the source of adenosine and its mechanism of release. Inhibition of the nucleoside transporter with dipipyridamole greatly enhanced adenosine release evoked by glutamate, NMDA, kainate receptor activation, and electro-shock of cortex-nucleus tractus solitarius with α,β-methylene ADP and GMP had no effect on either kainate- or AMPA-evoked adenosine release but decreased glutamate- and NMDA-evoked adenosine release by 23% and 68%, respectively. NMDA-evoked adenosine release, but not kainate- or AMPA-evoked release, was Ca2⁺-dependent. These results indicate that activation of non-NMDA receptors releases adenosine per se in a Ca2⁺-independent manner. In contrast, NMDA receptor activation evokes a Ca2⁺-dependent release of a nucleotide which is subsequently converted extracellularly to adenosine. Although neither NMDA- nor non-NMDA-evoked adenosine release occurs via the nucleoside transporter, this transporter does appear to be a major route for removal of adenosine from the extracellular space. (Supported by the MRC of Canada)

419.3

**BLOCK OF NMDA-EVOKED ADENOSINE RELEASE BY IBMX IS NOT DUE TO INHIBITION OF PHOSPHODIESTERASE I OR IV.** T.D. \*White* and C.G. Craig. Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

Activation of NMDA receptors in rat cortical slices evokes a Ca2⁺-dependent release of a nucleotide which is then degraded extracellularly to adenosine. To test whether the released nucleotide might be cAMP, we examined the effects of PDE inhibitors on adenosine release. IBMX (IBMX), a non-selective PDE inhibitor, blocks NMDA-evoked adenosine release but this was not accompanied by enhanced cAMP recovery in the medium. However, it seemed possible that cAMP might be degraded intracellularly to 5'AMP which is subsequently released to adenosine. To test this possibility, we measured the breakdown of cAMP to 5'-nucleotidase, PDE I, II and IV have been isolated in the cortex. Inhibitors of Ca2⁺/calmodulin-dependent PDE I (8-methoxymethyl-IBMX, W-7 and calmidazolium) and cAMP-specific PDE IV (rolipram) did not mimic the effects of IBMX on NMDA-evoked adenosine release. It appears that IBMX decreases the NMDA-evoked release of purines from cortex both by inhibiting PDE II or by acting at a site other than PDE. (Supported by the MRC of Canada)

419.4


Adenosine transport inhibitors have been suggested for the treatment of neuropsychiatric disorders. Heterogeneity of adenosine transporters has been described with differential sensitivity to dipipyridamole (DPR) and nitrobenzyl-xanthosine (NBI) in the brain of some mammals. Preparation of crude membranes from human parietal cortex and [3H]DPR as well as [3H]NBI radioreceptor assays were performed as described. [3H]DPR and [3H]NBI revealed approximately the same number of binding sites with a Bmax of about 100 fmol/mg protein and Ki's in the low nanomolar range. They displaced each other completely from their respective binding sites with Ki's equivalent to their respective Ki's. In conclusion, the data obtained so far in human brain makes the development of CNS-specific adenosine transport inhibitors on the basis of DPR- and NBI- sensitive transport unlikely.

419.5

**CHARACTERIZATION OF ADENOSINE UPTAKE SITES IN CULTURES OF CHICK EMBRYO RETINAL CELLS.** \*R. \*Pase-de-Carvalho* and E. \*Brassard*. Neurobiol., Univ. Fed. Fluminense, Niterói, RJ 24000, Brazil.

The presence of a specific uptake system for adenosine that can be inhibited by nitrobenzyl-xanthosine (NBI) or dipiridamole (DPR) was previously demonstrated in cultures of chick embryonic retina cells. Here we showed that (±)-NBI binds with high affinity to adenosine uptake sites on intact retinal cells. Cultures obtained by disassociation of 8-day-old chick embryonic retinas and incubated in BME with 5% fetal calf serum for 6 days at 37°C were washed and incubated in Hank's with 2-50uM (±)-NBI in the absence or presence of unlabeled NBI (10uM) to determine nonspecific binding. The binding was blocked approximately 70% in the presence of unlabeled NBI or DPR and attained equilibrium after 10 min at 37°C. The addition of NBI or DPR after equilibrium completely displaced specific binding, indicating the binding to surface sites rather than (±)-NBI uptake into cells. The data indicate that adenosine uptake sites labeled with (±)-NBI can be directly studied in living intact retinal cells and that primary cultures of chick embryonic retinal cells are excellent models for the study of these sites and their regulation by external factors.

419.6

**[3H]ADENOSINE TRANSPORT IN POSTMORTEM HUMAN BRAIN.** J.G. \*Guu*, G. \*Kalls*, and J.D. \*Geiger*. Dept. Pharmacology, Univ. of Manitoba, Faculty of Medicine, 700 Bannatyne Avenue, Winnipeg, Manitoba, R3E 0W3.

The kinetics of [3H]adenosine transport was characterized in cerebral cortical synaptosomes prepared from postmortem human brain. For this assay, it was determined that the adenosine transport inhibitors dimethylxanthine and diltiazem were instantaneously and completely effective in blocking adenosine transport. For 5 sec incubations, two kinetically distinguishable processes were identified; the Ki and Vmax values for high affinity adenosine transport were 89 nM and 0.98 nmoles/min/mg protein and for low affinity adenosine transport were 4.5 nM and 15.2 nmoles/min/mg protein. With incubation of 1 min [3H]adenosine accumulation was at 5 sec and 10 sec at 600 s; hence adenosine transport was not a concentrative process. For 5, 15, 30, 60 and 600 sec incubations, 12, 23, 34, 43 and 80% of transported [3H]adenosine was metabolized mainly to its phosphorolated derivatives AMP, ADP, and ATP. The concentration (μM) of total accumulated radiolabeled purines at these times was 0.3, 0.5, 1.0, 1.3 and 5.6, respectively; hence the accumulation of radiolabeled purines was constant only when extensive metabolism was present. In the presence of 10 μM EHNA, an adenosine deaminase inhibitor, and 10 μM toadalin, an adenosine kinase inhibitor, metabolism was 14, 14, 16, 14, and 38% of the transported [3H]adenosine and total radiolabelled purine accumulation was 0.3, 0.5, 0.5, 0.7, and 1.8 μM; hence concentrative accumulation of radiolabeled purines can be inhibited by the inhibition of adenosine metabolism. Thus, adenosine transport in human brain synaptosomes is via a facilitated diffusion system that exhibits low affinity for adenosine.

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Intracellular microelectrodes were used to characterize the adenosine receptors of 102 myenteric neurons. Biocytin was injected from the microelectrodes to characterize the morphology and sensitivity to adenosine receptor agonists. Superfusion of adenosine, the A2 agonists 5'-N-ethylcarboxamidoadenosine and CGS 21680 or the A1 selective agonist N6-cyclopentyladenosine and 2-chloro-N6-cyclopentyladenosine (CCPA) evoked an inhibitory response in 93 of 102 AH/Type II neurons (93.1%). The response consisted of membrane hyperpolarization, associated with a decrease in firing rate, increase in the hyperpolarizing after-potentials, inhibition of spike discharge and a decrease in cell input resistance. The latency profile for inhibition of cell input was CCPA > CGS 21680 > ADO (N=40); the EC50 for CCPA (15.1±2.17 μM) was 1000 fold lower than that of CGS 21680 (5.60 ± 2.54 μM). This effect was reversible with the A1 selective antagonist 8-cyclopentyl-1,3-dimethylxanthine (10-0.10-6 μM). Of 30 responsive neurons, 27 neurons (90%) had Dogiel Type II and 3 neurons (10%) had Filamentous morphologies. It is concluded that inhibitory A1 adenosine receptor exist mainly on myenteric AH/Doigiel Type II neurons. (Supported by NIH R29 DK44417 to FLC and ROI DK 37328 to JDW).


PC12 (GGt) is a putative transcription factor that regulates the synthesis of growth hormone (GH) and prolactin. Hormones and other agents regulate PI-3' kinase by various mechanisms, including effects on adenylate cyclase (AC). Since AC activity can be increased or decreased by a number of adenosine (Ado) via A1 or A2 receptors, respectively, we studied the effects of these ligands on mRNA synthesis under the control of the PI-3' promoter. GH-producing rat pituitary tumor cells (GH4C1) were transiently transfected with a chimeric reporter construct containing part of the 5'-flanking sequence of the PI-3' gene and linked to the coding sequence for chloramphenicol acetyl transferase (CAT). After 24h, various concentrations of forskolin (Fosk), a direct activator of AC, R-R-phenylisopropyladenosine (PIA, an A1 agonist), 8-cycloptensylnucleotide (CPT, an A3 antagonist) and/or Ado deaminase (ADA) were added. Cells were harvested 24h later and lysates were assayed for CAT activity. ADA altered both the magnitude and dose-dependence of Fork-stimulated CAT activity, suggesting a role for A1-Ado. In the presence of 2 U/ml ADA, the Forsk concentration giving half-maximal increase in CAT activity was increased 2- to 3-fold, and CAT activity was stimulated 25- fold by 0.5 μM Forsk. PIA (0.1 or 1 μM) consistently inhibited the expression of this construct to 40-60% of the basal value in the presence of 0.25 μM Forsk and 2 U/ml ADA. The effect of 0.1 μM PIA was completely blocked by 1 μM CPT. In the absence of ADA, responses to PIA were variable, e.g. 10 nM PIA stimulated construct expression 2-fold in some experiments. Our results indicate that adenosine should be added to the growing list of neurotransmitters and hormones which regulate pituitary hormone production at the transcriptional level. (Supported by NIH grant DK16638 and the Cohen Fund)


We have previously shown that specific adenosine A1 receptor activation in the rat medulla induces a motoneuronal excitability decrease and increases input resistance (RI). This decrease in excitability is due in part to an adenosine-mediated increase in afterhyperpolarization (AHP) amplitude. In order to understand better the mechanisms underlying A1 receptor effects, we recorded intracellularly from vagal motoneurons using a rat brainstem slice preparation before and during sub intrusion with either adenosine (10 μM-1 mM) or a specific A1 receptor agonist, cyclopentyladenosine (CPA, 10 μM). Synaptic blockade with either TTX or a permeant containing Mg2+ (10 μM), low Ca2+ (0.5 mM) blocked the CPA-induced increase in RI. Blockade of Ca2+ entry with 10 μM-3 mM Ca2+ abolished the CPA- and AHP amplitude, and increased excitability. Interestingly, no effect was seen with exogenous adenosine itself, unless adenosine uptake had been blocked with diphenhydramine (25 μM). We conclude that the CPA-induced RI increase, and possibly the AHP increase as well, is due to A1 receptor actions on presynaptic mechanisms, 2) the ion channel mediating the increase in AHP amplitude is sensitive to both but not 4AP or ampin, and 3) the ion current system in the vagal motor nucleus effectively buffers extracellular adenosine.


The accepted binding mode for a xanthine within the A1 receptor superimposes N1,N2,N3, and the N of the xanthine with the 4 identical xanthine base. This mode does not overlap the xanthine C6-N or the adenosine C8-R positions, the positions which impart potency and selectivity to xanthine-based antagonists and substituted adenosine agonists. With superimposition of N1,N2, and N of adenosine and N1,N3 and N of the xanthine, the C6 and C8 positions are in close proximity. MOL 102,234 (MDL) 1R-1,3-dipropyl-8,8-(1-phenylpropyl)xanthine) is a C8 substituted xanthine with the same stereochemistry as the C8-N phenylisopropyl substitution of the A1-selective agonist R-PIA. MOL 102,234 has Ks values at the adenosine A1 and A3 receptors and 0.86 and 0.53 μM, respectively. The compound is a very weak phosphodiesterase inhibitor with IC50 values against PDE Type III and PDE Type IV of 104 μM and 28.3 μM, respectively. This is 10,000-fold weaker than its potency at the A1 receptor. In guinea pig ileum, MOL 102,234 induced weak negative chronotropic and inotropic responses. At a concentration of 10 μM has no effect on heart rate or diastolic blood pressure. In conclusion, MOL 102,234 is a selective A1 antagonist and supports the new binding mode of xanthines to the A1 receptor.

SOEETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
419.13 
IN VIVO ACTIVATION OF AD1 RECEPTOR ENDOCYTOSIS BY CCQA 

The effect of 2-chloro-N6-cyclopentyladenosine (CCQA), the most selective A1, adenosine receptor agonist, was studied on 3'S-TBP binding measured "ex vivo" in the mouse brain. In fact, the increased decrease of 3'S-TBP binding in the rat and mouse brain homogenate reflect a reduction and an enhancement in the expression of the GABAa receptor-coupled chloride channel, respectively. CCQA (0.5 mg/kg i.p.), like negative modulators of GABAAergic transference, elicited a 30-40 min rise in the close-dependent increase of 3'S-TBP binding in the mouse cerebral cortex, hippocampus, striatum, but in the cerebellum. The effect of CCQA lasted for more than 4 hrs. Saturation studies revealed that the effect of CCQA on 3'S-TBP binding was abolished by the concomitant administration of the specific A1 receptor antagonist DPCPX (3 mg/kg i.p.). Moreover, apecsin (0.5 mg/kg i.p.), an anticoagulant and anticonvulsant benzodiacepine receptor ligand, completely abolished the increase of 3'S-TBP binding induced by CCQA. In spite of its capability of interacting with the site of GABA coupled chloride channel, CCQA (1-3 mg/kg) completely antagonized the convulsant activity of pentylentetrazol and iozanol, two GABA function inhibitors, while failed to antagonize kainic acid and strychnine-induced seizures.

419.15 
EXPRESSION OF ADENOSINE A1 AND A2 RECEPTOR SUBTYPES IN PRIMARY CULTURED NEURONS FROM FETAL RAT FOREBRAIN. J. L. Deval*, F. Nicolas, J. Billet and V. Koziel. INSERM U373 30 rue Lhomme, 75754 PARIS, FRANCE.

The expression of both adenosine A1 and A2 receptors, which are physiologically coupled to adenylyl cyclase via a G protein, was investigated by radioligand binding methods in primary cultured neurons isolated from fetal rat forebrain and grown in serum-free medium for 8 days. A1 receptors were labeled by incubating intact neurons in 50 nM Tris-HCl buffer (pH 7.4) for 120 min at 25°C with 2 nM adenosine deaminase and increasing concentrations of [3H]d,l-N6-cyclopentyladenosine (CCPA), using cytochrome-cytochrome c, for the determination of non-specific binding. A2 binding sites were analyzed by incubating the cells for 15 minutes at 25°C with 2 nM [3H]CGS 21680, a specific ligand. Both radioligand bound specifically and with high affinity to a single population of sites. Scatchard plots yielded Kd values of 2.9 nM (for [3H]CCPA, 1.7 nM for [3H]CGS 21680 and 33 nM for [3H]Forskolin. Maximum numbers of sites were 160 ± 14, 104 ± 15 and 254 ± 2 fmol/mg protein for CCPA, CGS 21680 and forskolin, respectively. The addition of 1 μM Gpp(NH)p, a GTP analogue, increased significantly Kd values for both CCPA and CGS 21680, suggesting that receptors are, at least partly, linked to G proteins.

419.17 

We have previously reported the expression of the human adenosine receptor (A2R) on human chromosome 22, suggesting an important role in motor function and extrapyramidal disorders. Complementary DNAs (cDNAs) for the A2R have been isolated from dog (B & B 173,1169) and rat (Mol Br Res in press). These A2D cDNAs exhibit substantial nucleic acid and amino acid homology in the transmembrane (TM) domains of the two species. To further understand the functional significance of sequence divergence at the carboxy terminus of the A2R, we have begun to characterize the human A2R gene. A panel of somatic cell hybrids containing various human chromosomes on a rodent background was screened with a 540 bp fragment of coding sequence for the rat A2R. Hybridization was detected with the cDNA 22 containing cell line. Localization on chromosome 22 proximal to a breakpoint at 22q11 was then established by hybridization with hemizygous somatic cell hybrids. A human chromosome 22 cosmid library was screened with the same probe and two clones were isolated. Southern analysis of one of the cosmid clones which selectively hybridized to the rat probe was subcloned and sequenced. The predicted amino acid sequence of this fragment is 89% homologous to the dog and 84% to the A2Rs (70% overall identity and greater than 90% identity in the transmembrane domains). We conclude that A2-like sequences homologous to the dog and A2 Rs reside on human chromosome 22.

419.18 

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420.1
NUCLEOTIDE RECEPTORS IN PHALEOCHROMOCYTOMA (PC12) CELLS. L. de Souza, S. Baba, A. Lange and J. K. Reed. Depts of Biochemistry, Zoology and Chemistry, University of Toronto, Brindille College, Mississauga, Ont. L5I 1S6.

The effect of extracellular ATP was studied in PC12 cells, a neurosecretory line that co-releases ATP with catecholamine upon stimulation. We have examined the effects of ATP by monitoring both cytosolic free Ca$^{2+}$ concentrations [Ca$^{2+}$]i and inositol phosphate (IP) levels. NMDA and kainic acid (KA) evoked a transient increase in [Ca$^{2+}$]i as measured with the Ca$^{2+}$-sensitive dye fura-2. The increase was eliminated by DTA. ATP and IP were not detected in PCs and ATP had little effect. A variety of ATP congeners were screened to identify the purinergic receptor subtype involved. The rank order potency of these analogues is not consistent with either P2X or P2Y purinergic receptors. The effect of ATP on IP levels was determined using [3H]-inositol IP levels increased in response to ATP and other nucleotides such as UTP and TTP, but not GTP. The rank order potency for agonist-induced stimulation of IP production followed closely the rank order potency for the calcium response. We propose that PC12 cells express a P2X nucleotide receptor that is distinct from classical purinergic receptors.

This research was supported by NSERC.

420.2

ATP increases intracellular Ca$^{2+}$ in glial cells from the spinal cord and other regions of the CNS by activating P2Y purinergic receptors. Signal transduction mechanisms of this effect of ATP were investigated by measuring Ca$^{2+}$1 in individual cells and delivering agents intracellularly by whole-cell patch clamp electrophoresis. Glia were studied in primary dissociated cultures prepared from the dorsal spinal cord of neonatal rats (2-14 days). Ca$^{2+}$1 was measured using the fluorescent dye, Fura-2. Cells were loaded with the dye by incubating with the cultures in the permeant form, Fura-2 AM (2 μM). The standard intracellular solution used for whole-cell patch clamp work and the solution stored in reagents and ATP had no effect on the response to ATP. These results lead us to conclude that ATP elevates Ca$^{2+}$1 in PC12 cells by a mechanism independent of the PC12 cell Ca$^{2+}$1 stores.

420.3

Intracellular recordings were made from myenteric neurons in vitro to study the actions of ATP applied by superfusion (10 nM-10 μM). ATP showed pre- and postsynaptic actions in a concentration-dependent manner. ATP produced a membrane depolarization associated with an increase in input membrane resistance in 5 cells and a hyperpolarization accompanied by a fall in input resistance in 1 cell. Both responses were reversed in polarity near the K$^{+}$ equilibrium potential and were markedly reduced or abolished in Ca$^{2+}$-free/high Mg$^{2+}$ solution. These responses suggest that ATP may interact with K$^{+}$ currents and the ATP-induced depolarization and hyperpolarization were nullified by manual clamp method. It should be added that ATP at lower concentration (<100 nM) did not affect ACh-synaptic release of ACh. Thus, it is concluded that ATP may act pre- or postsynaptically or both and may regulate synaptic transmission in the myenteric plexus. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Japan.

420.4

Receptor-mediated cytosolic Ca$^{2+}$ mobilization studies readily adapted in the rat parotid acinar cell model, where at least five different neurotransmitter receptors participate in Ca$^{2+}$ elevation. Previously, we observed that extracellular ATP at high concentrations (10 μM) stimulated a Ca$^{2+}$ influx without IP formation in cell suspensions, and that the concentration-response curve was biphasic. Fluorescence microscopy ratio imaging methods were used to study ATP-induced calcium responses in perfused, single acinar cells loaded with Fura-2. All cells (93%) which respond to muscarnic and Substance P activation through the same metabotropic receptor, responded to ATP. Analysis of the concentration-response curves showed that two responses to ATP could be resolved in most cells. The high affinity response, detectable at 0.1 μM ATP, saturates at 1-5 μM and rapidly desensitizes in 50% of the cells, fully recovering within about 6 min after washout. At much higher concentrations of the ATP (300-600 μM), a large, more slowly developing Ca$^{2+}$ rise occurs, which can be blocked by Mg$^{2+}$ as expected for an ATP receptor specific response. The latter response does not desensitize, and is difficult to fully reverse on washout, possibly because a large influx of extracellular Ca$^{2+}$ is handled differently from an equally high Ca$^{2+}$ level produced through muscarinic receptor activation, which is readily and rapidly reversed. Examination of these two Ca$^{2+}$-mobilizing purinergic receptors reveals the participation of different mechanisms of Ca$^{2+}$ homeostasis in recovery of resting Ca$^{2+}$ levels in individual cells.

420.5

Adenosine triphosphate (ATP) is released from nerve terminals together with neurotransmitters such as acetylcholine. Aside from its action on neural receptors, other functional aspects of extracellular ATP are not yet known. Since extracellular ATP has been implicated in the disruption of intracellular Ca$^{2+}$ homeostasis, we examined the effect of ATP on the Ca$^{2+}$ transport system. Results indicated that ATP at 1-2 mM enhanced the voltage-dependent calcium influx (VDCl). The enhancement of VDCI activity was specific for ATP and activity was blocked by ADP and AMP. Furthermore, the slowly hydrolyzable ATP, ATPyS, inhibited synaptosomal ATPase and an increase which ATP hydrolysis was involved in ATP-induced enhancement of VDCI. Under similar conditions a 43 kD protein was phosphorylated by an ecdy-kinase. The level of phosphorylation of this protein was dependent on exogenous calcium and was dephosphorylated upon depolarization. It is possible that the 43 kD protein is functionally linked to the ATP-induced VDCI activity.

420.6
GUANOSINE AND GTP ENHANCE NGF-STIMULATED NEURITE OUTGROWTH IN PC12 CELLS. John W. Grabois and Michael S. Puthiyaveetil. Departments of Biomedical Sciences and Medicine, McMaster University Health Sciences Center, Hamilton, Ontario, Canada L9N 1S5.

Extracellular guanosine or GTP (30-300 μM), but not adenosine (0.3-300 μM) enhanced the neurogenic effect of NGF in rat phaeochromocytoma (PC12) cells. None of these purines induced significant neurite outgrowth in the absence of NGF. The effects of guanosine and GTP were synergistic with that of NGF in increasing the proportion of cells bearing long neurites. Adenosine receptor agonists such as 5'-N-cyclopentyladenosine (CPA), 5'-N-ethylcarboxamidoadenosine (ECA) or 5'-N-ethylcarboxamidoadenosine (NECA), also increased NGF-stimulated neurite outgrowth, but not to the same extent that did guanosine with NGF. High concentrations (1 μM) of CPA (1-3H)-diethylpyroili-8-2-amino-4-chloro)-theanine, a putative adenosine receptor agonist, blocked the synergistic effect of guanosine and NGF. However, adenosine $A_2$ receptor agonists DPX (1,3-dipropylxanthine) $A_3$ receptor agonists and $A_7$ receptor agonists did not inhibit the effects of guanosine plus NGF. Interestingly, guanosine also acted synergistically with NGF and adenosine $A_2$ receptor agonists, and NGF alone to induce significant neurite outgrowth in the absence of NGF, although neither of these compounds alone stimulated significant neurite outgrowth in PC12 cells. These data indicate that guanosine may be a co-receptor for the peripheral adenosine $A_2$ receptor to enhance neuritogenesis. Moreover, neither ADP and GTP (purinergic $P_2$ receptor agonists) or ATP (purinergic $P_2$ receptor agonists) interfere with the neurogenic effects of ATP and neurite outgrowth of PC12 cells. Support: Hospital for Sick Children Foundation, Toronto, ON. N.W. is a Neuro-Endocrinology Foundation Research Fellow.
411.5


When stimulated with elevated K+, slices of hippocampal area CA1 release glutamate and asparate in a 5:1 ratio. Release of these amino acids from CA1 slices originates predominantly from the Schaffer collateral-commissural projection and CA2 hippocampal area. Analyses of the pathways that lead to the release of glutamate and asparate reveal that release is mediated by glutamate receptors and asparate release is partially dependent on asparate release. These results indicate that extracellular glutamate terminals can release asparate, even under conditions where membrane transport of released amino acid is expected to be minimal. (Supported by NIH grant NS 16064.)

411.4

EFFECT OF DRUG-INDUCED CHANGES IN THE DISPOSITION AND METABOLISM OF NEUROTRANSMITTER ON THE QUANTITATION OF 3H-SEROTONIN (SHT) RELEASE. F. Morice, D.L. Smith, B.K. Oakes, D.L. Smith and B. Oakes, Dept. of Anesthesiology, West Virginia University Health Sciences Center, Morgantown, WV 26506 and Department of Psychiatry, McGill University, Montreal, Canada

While evaluating the effect of 2-methylpropanoyl (2-Me-cAMP) on the release of 3H-SHT from rat spinal cord tissue, it was determined that the drug altered the intracellular disposition of the neurotransmitter. Suppression of the tissue with 2-Me-cAMP resulted in a concomitant decrease in the 3H-SHT efflux which occurred without a concomitant elevation in the efflux of 3H-SHT. This effect was blocked by the co-suppression of an inhibitory GABA receptor, suggesting the drug disrupts intracellular storage of neurotransmitter following active uptake. The effect of 2-Me-cAMP on the 3H-SHT efflux was slow in onset. Fifteen minutes were required before increases in 3H-SHT efflux could be detected. However, this length of drug exposure is frequently achieved in the course of routine neurotransmitter release assays. Therefore, if total 3H-SHT is quantitated rather than separated neurotransmitter and metabolite levels, it is clear that drug-induced increase in the metabolite efflux could be mistakenly associated with an enhancement of depolarization-evoked 3H-neurotransmitter efflux. For example, when calculated using total 3H-SHT, 10 µM 2-Me-cAMP can cause a apparent 3 fold enhancement above the control (no 2-Me-cAMP) response to 15 nM K+ stimulation. On the other hand, when superfuse fractions were analyzed for 3H-SHT and 3H-SHT content, K+ evoked increases in 3H-SHT efflux were comparable in drug treated and control conditions. However, drug-induced increases in 3H-SHT efflux were of the same magnitude as those seen from tissues which were not exposed to elevated K+ concentrations. These results indicate that the separation of transmitter from metabolites is necessary to ensure that drug-induced changes in the disposition of neurotransmitter do not adversely affect the assessment of radiolabeled neurotransmitter release evoked by depolarization induced mechanisms.

411.6


Some of the excitatory glutamate pathways in the rat hippocampal formation, including the Schaffer collateral-commissural fibers and the lateral perforant path, take up proline by a high affinity Na+-dependent process. The role of proline in glutamate transmission is unknown. This study compared the uptakes of proline and glutamate by crude synaptosomal preparations of the hippocampal formation during ontogenesis. Uptake of both amino acids was determined from the same tissue samples. At all ages studied from 9 ond, glutamate was taken up by a single high affinity, Na+-dependent process (Km = 5 µM). In contrast, proline uptake was taken up by both high affinity (Km = 5 µM) and low affinity (Km = 100-1000 µM) Na+-dependent processes. High affinity uptake of proline increased 4-fold and high affinity uptake of glutamate increased 2.5-fold between 9 and 15 d after birth. At 15 d postnatal age, uptake had reached adult values and it remained relatively constant afterward. In contrast, proline uptake remained elevated above adult values during the 15-21 d period. These results demonstrate that high affinity uptakes of proline and glutamate, which are co-expressed by many of the core cell pathways, develop during the same postnatal period. The developmental overshoot in proline uptake suggests that this process may play a more significant role in synaptic physiology during adolescence in the rat than during adulthood. (Supported by NIH grant NS 16064.)
421.7

EFFECTS OF ACUTE AND CHRONIC INSULIN TREATMENT ON NORADRENERGIC (NE) UPTAKE AND NE TRANSPORTERS IN RAT BRAIN AND PC-12 CELLS. D. F. S. Dermot Reynolds*, P. Slabas, D. Stone, and J. Payne. Dept. of Psych Sci. Med. and U. Washington, Seattle, WA 98195 and VA Medical Center, Seattle, WA 98108. Previous work from this laboratory has led to the suggestion that insulin (INS) receptors in the CNS may be located presynaptically on noradrenergic neurons. To test whether insulin may serve as a neuromodulator for NE neurons, we measured 3H-NE uptake into hypothalamic slices, and into the rat pheochromocytoma PC-12 cells. Acute INS treatment significantly inhibited 2 min NE uptake into hypothalamic slices and into PC-12 cells over a concentration range of 0.1 - 10 nM, in good agreement with the Kd of the insulin receptor (-318±13 nM and -29±8% inhibition, respectively, with 10 nM insulin). Chronic INS infusion in vivo resulted in a significant decrease of NE transporter mRNA levels vs vehicle-treated controls (brain area=1235.7±265 vs 1282.4±292). These results suggest that INS may play a physiological role in the acute and chronic regulation of synaptic NE concentrations via modulation of its re-uptake within certain populations of NE neurons in the CNS.

421.8


The nucleoside adenosine has a wide range of physiological effects including modulation of CNS neuronal activity. Adenosine effects are terminated primarily by uptake, via the nucleoside transporter, from the extracellular fluids into the cell. Inhibitors of the nucleoside transporter are able to increase extracellular adenosine levels, and thereby potentiate the effect of endogenously released adenosine.

R7531, a nitromide derivative, has been reported to be unique as an extremely long-lasting, tightly-binding inhibitor of nucleoside transport (Van Belle and Jansen, Nucleosides and Nucleotides, 18:975, 1991). We have examined the nature of R7531 interaction with the nucleoside transporter in rabbit CNS cortical synaptosomes, using [3H]bretrolylthionine ([3H]BNPMP) as a specific probe. Synaptosomes were incubated in the absence and presence (1mM R7531 or 1mM benzoylthionine) of 45 min, after which, an aliquot of each was removed for analysis of [3H]BNPMP (0.4mM) binding. The remaining synaptosomes were washed (10 min centrifugation at 40 000g). 


diversity was washed and rinsed once with 200 uL buffer - before washing, it was washed for a further 2 washes, whereas the inhibition of [3H]BNPMP binding by R7531 after 5 washes (+5%) was not significantly different from that observed before washing, but did not inhibition of either 10mM adenosine or 10mM benzoylthionine. This result may be due to our 3D model, which suggests that the nucleoside transporter is the nucleoside transporter in rabbit CNS cortical synaptosomes.
422.1 DELAYED INCREASE OF EXTRACELLULAR ARGinine (ARG), THE NITRIC OXIDE (NO) PRECURSOR, FOLLOWING ELECTRICAL WHITE MATTER STIMULATION IN CEREBRAL SLICES. C. Hansel1, A. Batchelor2, M. Cuencod1 J. Garnythe5, T. Knöpfel2 and K.Q. Do1.1 Brain Res. Inst. Univ. of Zurich, Switzerland; 2Dept. of Physiology, Univ. of Liverpool, U.K.

Amino acids were measured in perfusates from L-shaped rat cerebral slices installed in a Krebs-filled three-compartment system following electrical white matter stimulation. The lateral compartments housed white matter and a cortical section containing parallel fibres respectively, whereas the central compartment housed cortical structures at the point of bending. This arrangement allows electrical stimulation while the perfusion medium passing the central chamber can be collected for amino acid analysis with HPLC. Following a 2-minutes stimulation period of either 2 Hz (n=6) or 6 Hz (n=5) Arg levels were significantly raised above levels already present in the Krebs-solution (5.5±1.75 pmol/min respectively 7.3±1.26 pmol/min). Arg is the precursor of NO, a neuronal messenger in the brain, which is synthesized by NO synthase. Since Arg and the NO synthase are located in different cell types it can be suggested that Arg passes through the extracellular space in order to replenish the precursor pool for NO synthesis.

422.3 NITRIC OXIDE SYNTHASE-CONTAINING CELLS IN THE CEREBRAL CORTEX OF RATS. B.J. Wodicka1, L.G. Vathalond2, Y.N. Kharas1, M. Nakanishi1, H.U.P. Schmidt1 and A. Roth1.1 Dept. Cell Biology & Anatomy, UNC, Chapel Hill, NC 27514, USA; 2Dept. Vascular Surgery, Univ. of Texas, Shreiner B. Austin Post Grad. Med. Sch., IL 60604; and 3Dept. Pharmacology, Northwestern U., Chicago, IL 60611.

We have employed histochemistry for NADPH diaphorase and immunohistochemistry for the nitric oxide metabolite nitrite to detect nitric oxide synthase (NOS-1) to reveal neurons and fibers in the rat cortex likely to synthetise nitric oxide. N-terminal-amino acidized animals were perfused with mixed aldehydes; Vibratome sections 20-100 μm thick were stained for NOS. The distribution of NOS-stained cells was plotted; detailed drawings showing their morphology were made with camera lucida. Particular attention was focused on neurons in the upper cortical layers.

NOS-positive neurons comprised about 0.5% of neurons in 5 ml. They were densest in layers 2, 3 and 6 and in the subcortical white matter. Stained neurons were morphologically different, but did not include typical pyramidal neurons. Most had axonless dendrites and axons branching close to the soma. Stained axons had predominantly radial orientation. Boutons were visible throughout the cortex, densest in layers 3-4 and 6. The morphological impression that NOS-positive neurons are local circuit neurons was supported by double-label experiments in which injections of CTB-gold or WGA-HRP were placed in contralateral cortex, thalamus, caudoputamen or spinal cord. In all cases, retrogradely labeled neurons and NOS-positive ones belonged to separate populations. In tangential sections through the barrel field NOS-positive neurons and dendrites were confined to the septa between barrels. Presenting our results, we postulate a role for immunocytochemistry in combination with NADPH diaphorase histochemistry demonstrated that a large fraction of NOS-positive soma also contained GABA.

422.4 NITRIC OXIDE GENERATION RELEASES ('H)-NOREPINEPHRINE FROM HIPPOCAMPAL SLICES. K.M. Johnson and G. Lorentz. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555-1031.

Nitric oxide (NO), a diffusible second messenger, has suggested to potentiate neurotransmitter release. Cross-chopped hippocampal slices (300-300 μm) were prepared from male Sprague-Dawley rats, washed and incubated with ['H]-norepinephrine ([3H]-NE) for 30 min in Krebs bicarbonate buffer, containing 10 μM pargyline and saturated with 95% O2:5% CO2 (9:5). Efflux of ['H]-NE was terminated and the data are expressed as fractional release. Hydroxyamphetamine (300 μM), which is known to generate NO subsequent to cellular metabolism, induced a concentration dependent increase in the basal ['H]-NE release. The EC50 value was about 30 μM and 100 μM hydroxyamine produced a maximal 10-fold increase in the basal ['H]-NE release. 10 μM hydroxyamine increased basal ['H]-NE release about 2-fold. Since NO stimulates the activity of guanylate cyclase, we tested the effect of methylene blue, an inhibitor of this enzyme. 3 μM methylene blue alone induced a steady 2-fold increase in the basal ['H]-NE release and significantly potentiated the response to 10 μM hydroxyamine. 20 min pretreatment with 3 μM or 10 μM hydroxyamine did not significantly influence NMDA (300 μM) stimulated ['H]-NE release, while higher concentrations had inhibitory effects. Ongoing experiments are being carried out to characterize the mechanism underlying the effects of hydroxyamine and methylene blue in this preparation. Supported by DA-02073.

422.5 AN NMDA/NITRIC OXIDE PATHWAY MEDIATES LIGHT RESPONSIVENESS BY THE SUPRAESACHASMATIC NUCLEUS. S. Amat. CSIN. Concordia University, Montreal, Quebec, Canada.

The messenger molecule nitric oxide (NO) is produced in brain on stimulation of N-methyl-D-aspartate (NMDA) receptors, and receptors for NMDA have been implicated in light to the hypothalamic suprachiasmatic nucleus (SCN), site of a circadian pacemaker. We assessed the involvement of the NMDA/NO pathway in SCN responsiveness to light by blocking NMDA receptor or NO production in the SCN and evaluating the effect on light during photic stimulation in dark-adapted urethane-anesthetized rats. Photic stimulation (300-300 μm) 3 min) caused a rapid increase in light (ΔA = 37.3±4.4 pm, t=0.8). This effect was blocked by prior infusion of a competitive NMDA antagonist (CPP, 20 nmol) in the SCN (ΔA = 12.5±1.6 pm, t=0.01). Infusion 2 mm dorsal to the SCN had no effect, suggesting a role for NMDA receptors specific to the SCN. Infusion a competitive blocker of NO synthesis, Nω-nitro-L-arginine methyl ester (L-NAME, 40 nmol) in the SCN, but not 2 mm dorsal to the SCN, blocked the rise in light to in response to light (ΔA = 16.1±1.8 pm, t=0.01). This effect was selective and stereospecific: addition of L-arginine (80 nmol), which competes with L-NAME for the substrate site on the NO-generating enzyme, reversed the effect of L-NAME (ΔA = 42.1±9.8 pm, t=8). and infusion of an inactive isomer of L-NAME, D-NAME (40 nmol), had no effect (ΔA = 38.8±4.6 pm, t=12). The ability of light to stimulate heart rate and of infusion of a competitive NMDA antagonist or a competitive blocker of NO production in the SCN region to inhibit this effect suggests a functional link between activation of an NMDA/NO pathway and SCN light responsiveness.

422.6 EVIDENCE THAT NITRIC OXIDE (NO) MEDIATES THE CYCLIC GMP RESPONSE TO SYMPATHETIC ACTIVITY IN THE RAT SUPERIOR CERVICAL GANGLION (SCG). Marion L. Husberg, Hong Shen, Ferial Husn, and Clark A. Briggs.1 Neuroscience Research and Signal Transduction, Abbott Laboratories, Abbott Park, IL 60064.

Preganglionic stimulation increases cyclic GMP levels in the SCG. If it is not known what neurotransmitter mediates this response. However, exogenous nitroprusside and azip, which liberate NO, also have been shown to stimulate cyclic GMP synthesis (Yoshie & Oppenheim, 1983). We have measured whether NO is the physiological mediator of the cyclic GMP response to preganglionic stimulation.

Pared SCG were isolated and superfused in vitro at ambient temperature (21-23°C) with oxygenated Locke’s solution containing 0.3 mm 3-isobutyl-1-methylxanthine. Various drugs were applied for a period of 75-85 minutes before stimulation. In each pair, one SCG served as a non-stimulated control and the other received a preganglionic stimulation of 10 Hz for 30 sec. Cyclic GMP was determined by radioimmunoassay.

Upon stimulation, cyclic GMP levels increased 8-fold and the response was Ca2+ dependent. The NO synthase inhibitor, Nω-nitro-L-arginine (L-NNA), inhibited the cyclic GMP response in a concentration dependent manner which was partially reversed by the NO donor SNAP (1 mm). L-NNA (10 μM) blocked the response and this effect was stereospecific because D-NNA (10 μM) showed no inhibition. Additionally, the cyclic GMP response was inhibited by 65% by 10 μM L-NNA and a similar substance mediated, in an extracellular fashion, the cyclic GMP response to sympathetic activity in the rat SCG.
422.7 TRANSCRIPTION OF THE BRAIN NITRIC OXIDE SYNTHASE GENE IN NEURAL CELL CULTURES. D. Ming-Golomb & J.P. Schwartz*. Clinical Neuroscience Br., NINDS, NIH, Bethesda, MD 20892.

Nitric oxide synthase (NOS) generates the second messenger nitric oxide from arginine. The brain NO system, in cerebellar and accessory olfactory bulb, now present data on the expression of the NOS enzyme in cerebellar granule neurons prepared from postnatal day 8 rat pups, after 10 days in culture. Using specific oligonucleotide primers, we obtained reverse transcription of GCC RNA followed by polymerase chain reaction (RT-PCR) produced a 366 bp fragment, the expected size. Identity was confirmed by hybridization of a blot containing the electrophoresed product with a full-length cDNA probe for NOS, followed by high stringency washes (0.1XSSC-65°), which showed a positive signal for the same 366bp band. NOS activity was also detected in GCCs, by measuring conversion of arginine to citrulline, thus showing that GCCs express the brain NOS.

Preliminary results suggest that type 1 astrocytes are the predominant source of NOS mRNA and activity. These culture systems are being used to assess the modulation of NOS in cerebellar neural cells both in physiological and neurotoxic conditions.

422.8 CARBON MONOXIDE AS A PHYSIOLOGICAL MESSENGER INDICATED BY HEME OXYGENASE LOCALIZATIONS AND CYCLIC GMP REGULATION. David J. Hirst*, A. Verma, C. Olt, G. Bennett and S.I. Snyder. Dept. of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Abundant evidence indicates that nitric oxide(NO) is a major physiological molecule, accounting for endothelial derived relaxing factor(EDRF) of blood vessels, the tumorid and bacterial action of NO as an antibiotic, and its possible role as a neurotransmitter in the central and peripheral nervous system. The notion that a normally noxious gas such as NO might serve as a physiological messenger is further supported by the observation that NO mediates cell responses similar to those on other gas molecules (NO, CO). This is the first known biological role of CO(NO) as a physiological messenger. Carbon monoxide(CO) is endogenously generated by the enzyme heme oxygenase. Heme oxygenase was studied in concordant with cycloheximide P450 reductase, catalyzes the conversion of heme into biliverdin with the concomitant release of CO. We thought that this CO could function in an analogous manner to NO. The brain has been previously reported to have high levels of HO. We localized the constitutive form of this enzyme, in situ hybridization. Highest densities of HO mRNA are evident in the olfactory neurons of the olfactory epithelium as well as the olfactory neuronal layer of the olfactory bulb along with the granule cells in the bulb. We also observed discrete localizations in the cerebellum, hippocampus, pontine nucleus, olfactory bulb, and tuberence. It is probable that the physiological role of CO we utilized a primary culture of olfactory neurons which expressed HO at high levels as revealed by the in situ hybridization. These neurons have very high levels of CO receptor binding. CO receptor binding is specific for CO, which is not present in the cultures. These findings suggest that CO might be a messenger molecule similar to NO.


Striatal neurons express high adenylyl cyclase activity and large amounts of a stimulatory G protein, but very low levels of G protein mRNA. We have previously presented evidence suggesting that the novel stimulatory G protein Golf is expressed in the basal ganglia. In support of this hypothesis, we have now used synthetic oligonucleotides, derived from the published Golf cDNA sequence, to identify and characterize four independent Golf cDNAs from a rat brain cDNA library. These code for a protein of 381 amino acids with a predicted molecular mass of 44.3 KD, whose sequence is identical to the Golf sequence originally derived from a rat striatal epithelial library, except for one conservative amino acid substitution. Thus Golf is expressed in rat brain. To determine the cellular localization of Golf in the brain in situ hybridization and immunohistochemistry were performed. Golf mRNA was expressed in neurons in the striatum, nucleus accumbens and olfactory tubercle. For the immunohistochemical localization of Golf, rabbits were immunized with a synthetic peptide corresponding to amino acids 73-87 of Golf, conjugated to KLH. A rabbit polyclonal antibody that upon affinity-purification, specifically labelled a striatal protein of about 46 KD in Western blots. In immunohistochemical studies, this antibody selectively stained many neurons within the striatum, nucleus accumbens and olfactory tubercle. In addition, a stained fiber was seen leaving the striatum, and forming terminal networks in the globus pallidus, entopeduncular nucleus and substantia nigra pars reticulata. In conclusion, striatal projection neurons express a novel stimulatory G protein, Golf, which may couple the G proteins to adenyl cyclase.

(Supported by The Parkinson Foundation of Canada)

422.10 CHARACTERIZATION OF THE HIGH-AFFINITY FORSKOLIN BINDING SITE IN THE RAT STRIATUM. S.R. Vincent* & B. Phillips. Kinsman Laboratory of Neurological Sciences, Department of Psychiatry, The University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The diester forskolin is a potent activator of adenylyl cyclase. We have characterized the high-affinity binding site for [3H]forskolin in the rat striatum using quantitative autoradiography. Saturation analysis revealed a single high-affinity binding site with a Kd of 26 nM and a Bmax of 694 fmol/mg tissue. Lesion studies indicate that these binding sites are largely present on striatal neurons, including the striato-nigral, medium-spiny, projection neurons which contain D1 dopamine receptors. The abilities of various forskolin analogues to displace high-affinity [3H]forskolin binding correlated well with their effectiveness in activating adenylyl cyclase. The density and affinity of the striatal binding sites were decreased if [3H]forskolin was absent from the incubation medium. This is consistent with the hypothesis that binding of forskolin to the adenylyl cyclase involves an interaction with a stimulatory G protein. However, pretreatment of rats with SCH-23390, which blocks D1 dopamine receptors, and should therefore largely reduce the endogenously blocked stimulatory G proteins in striatal neurons, had no effect on forskolin binding. Similarly, pretreatment with the atypical neuroleptic clozapine, a potent inhibitor of dopamine-stimulated adenylyl cyclase, was without effect on high-affinity forskolin binding in the striatum. These data suggest that endogenous D1 receptor activation does not account for the very high levels of forskolin binding present in the striatum.

(Supported by the B.C. Health Research Foundation.)

422.11 P2X, PURINOCCEPTOR-MEDIATED PHOSPHOSCHONITRIDE TURNOVER, Ca2+ INFLUX AND HOMOLOGOUS DESENSITIZATION IN C6-GliaLMA. W.M. Lee, D. Ko, G.P. Lai, H. Huang, H.R. Chen & S.K. Lee. Inst. of Cell and Developmental Biology, National Taiwan Univ. Taipei, Taiwan and 1 Biological Psychiatry Branch, NIMH, MD 20892.

In response to ATP, C6-glioma cells accumulated inositol phosphates and elevated [Ca2+]i dose-dependently with an EC50 of 60 nM and 10 nM, respectively, for stimulation of phosphoinositide (PI) turnover. The order of potency of adenine nucleotides was ATP>>ADP>>AMP, adenosine, adenosine, a, b-ATP, a, b-ATP, adenosine, amp, a, b-ATP, ATP-stimulated PI metabolism was found to be partially dependent on [Na+]o and [Ca2+]i, but resistant to tetrodotoxin, amiloride, ouabain and inorganic Ca2+ blockers. In Ca2+-free medium, ATP caused only a transient increase in [Ca2+]i as O observed to a sustained [Ca2+]i increase in normal medium. The ATP-induced elevation of [Ca2+]i was resistant to Na+ depletion, verapamil and nifedipine, but was attenuated by La3+. The ATP-induced PI hydrolysis showed homologous desensitization following agonist preincubation and was unaffected by PEC inhibitors (stauroporine, H-7 and depletion of PEC activity. On the contrary, the inhibition caused by BIM or octilindolactone was inhibited by staurosporine, H-7 and polyoxyl 8. Our results suggest that P2X purinoceptors are coupled to PEC turnover and Ca2+ influx in C6-gliaLMA and its desensitization does not involve PEC activation.

422.12 FACTORS INFLUENCING THE EFFECT OF CALRETININ ON PHOSPHORYLATION OF A 39 kDA MITOCHONDRIAL PROTEIN FROM RAT BRAIN. L. Mahaffey & M. Jacobowitz. Lab. of Clin. Sci., NIMH, Bethesda, MD 20892.

The calcium binding protein calretinin (CR, 100 to 1000 nM) produced a significant reduction in the phosphorylation of a 39 kDa band in mitochondrial membranes. This 39 kDa band was maximally phosphorylated within 15 min while the inhibition of calretinin was most apparent after 2 min. Both the phosphorylation of the 39 kDa protein and inhibition by calretinin were pH dependent. The 39 kDa band was phosphorylated in the presence of 5 mM EDTA and a stimulation was observed in the presence of Mg2+ (4.5 or 10 mM) or 4.5 mM Mn2+, Ni2+, Ca2+ or Co2+. The inhibitory effect of calretinin was increased in the presence of Mg2+, Ca2+ and Ni2+ and was attenuated by 5 mM EDTA. Zn2+ (4.5 mM) inhibited both the phosphorylation of the 39 kDa band and the inhibition of phosphorylation protein. Calretinin also produced a slight inhibition in the phosphorylation of a 42 kDa band in the presence of 4.5 mM Ni2+, Co2+ or Ca2+. This band was tentatively identified as the alpha subunit of pyruvate dehydrogenase based on immunoblot and comigration with purified phospho-enzyme. These results suggest a possible functional role of calretinin in modifying mitochondrial enzyme activity through effects on protein phosphorylation.
422.15  
PURIFICATION AND CHARACTERIZATION OF BOVINE FOREBRAIN CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II M. Tau *  
R. Borviet, J.C. Wyman, Department of Neurobiology & Anatomy, University of Texas Southwestern Medical School, Dallas, TX 75390  
Calcium/calmodulin-dependent protein kinase II (Ca2+/CaM kinase), is an abundant brain protein proposed to mediate Ca2+/CaM-regulated signal transduction in neuronal tissue. The enzyme purified from rat brain is reported to exist as two isoforms (M, 550,000 & 615,000) that vary in their relative ratio of three subunits, M, 50,000, 58,000, and 60,000.  
In this study we have purified Ca2+/CaM kinase from bovine forebrain. The tissue was frozen in liquid N2 within 5 min of the animals death. The purification revealed two isoforms with apparent Ca2+/CaM protein properties based on the phosphorylation of known Ca2+/CaM substrate peptide (MBP/DTT). The two isoforms have similar purification characteristics, subunit composition, and physical properties. Native gradient-gel electrophoresis provided an estimated M, 500,000 for the major isoform. The autophosphorylated subunits of the major kinase have apparent M, 72,000 and 39,000 while those of the minor kinase are M, 72,000 and 53,000. Both bovine kinases bind calcium with high affinity and have mononuclear ant-ri-Ca2+/CaM kinase Ig-like domain. The major kinase exerts preferential cross-effects with monoclonal anti-Ca2+/CaM kinase IgG in a spot blot assay while the minor kinase does not cross-react at all. Western blotting technique using polyclonal anti-rat Ca2+/CaM kinase IgG shows that a M, 72,000 subunit of bovine kinase cross-reacts with the antibody. These results suggest that although the two isoforms of bovine kinases are similar to rat Ca2+/CaM kinase, there are several important differences between the rat and bovine enzymes. Supported by USDA NS 11061.

422.17  
The sigma receptor has been well characterized in terms of ligands that bind to it, but in function it may be related to the regulation of neurotransmitters. The sensitivity of the sigma receptor to 1) identify its presence in neuronal tissue and 2) test its potential role in the arachidonic acid (AA) cascade in these cells.  
Cerebella of eight-day-old neonatal rats were dissected and chopped into pieces. Cells were mechanically and enzymatically dissociated, subjected to differential centrifugation, resuspended, and plated on poly-D-lysine coated dishes. Cultures were tested with 10 μM, sodium arachidonate to inhibit growth of non-neuronal cells. Cells were allowed to develop for at least eight days in culture (DCE) before use in any assay.  
Sigma receptors were identified by the specific binding of 2 nM [3H]haloperidol in the presence of spiperone to inhibit labeling of dopamine receptors. The sensitivity of binding to 1) identify its presence in neuronal tissue and 2) test its potential role in the arachidonic acid (AA) cascade in these cells.  
Cells were harvested with Trizma buffer containing 1 μM (3H)arachidonic acid (3H)AA overnight, then washed to remove residual, unaccumulated labeled eicosanoid. The release of [3H]AA was stimulated by 50 nM NMDA, resulting in the release approximately 0.5% of total accumulated [3H]AA. About 30% of stimulated release could be inhibited by 10 μM (+)-pentazocine. Collectively, these findings provide the first evidence that sigma receptors reside on cerebellar granule cells, and that they may be involved in the regulation of the release of arachidonic acid in these cells. This is the first direct association of sigma receptors with this metabolic second messenger system.

422.18  
ARACHIDONIC ACID METABOLISM MAY INVOLVE VOLTAGE-SENSITIVE COMPONENTS IN ACUTELY DISSOCIATED EMBRYONIC CHICK SPINAL CORD CELLS. K.S. Middaugh, A. Prato, S.V. Smith and G.D. Langle, Laboratory of Neurophysiology and Instrumentation and Computer Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892  
Prostaglandin E2 and most other types of eicosanoids are metabolites of arachidonic acid (AA) formed by cyclooxygenase (COX) and the lipoxygenase (LOX) pathway. Eicosanoids are produced by cells in response to various stimuli. The effects of several of these inhibitors on the steady-state membrane potential of individual chick cells using two different electrophysiological approaches. Flow cytometry coupled with a voltage-sensitive oxonol dye monitored the effects of bath applications on populations of cells in suspension. Conventional whole cell patch techniques for intracellular voltage recordings measured the effects of superfusing single plates of cells that were not stained with the oxonol dye. We found that four molecular different agents, indomethacin, ibuprofen, 15-15a-prostaglandin J2 (a-15-15a-cycloxygenase inhibitor), BW-535C and NDGA (nordihydroguaiaretic acid) decreased the intensity of oxonol signal and hyperpolarized cells at concentrations known to block cyclooxygenase activity. Subsequent exposure to PGE1 did not reverse these effects unless preparations were first depolarized by elevating extracellular potassium ion concentration. Then the PGE1 increased the hyperpolarization elicited by the drugs. These findings support the possibility that PGE1 acts through an intracellular mechanism which is normally prevented by extracellular calcium and/or influx. These gradients would also promote its net efflux after de novo syntheses.

We have shown previously that, in membranes prepared from female rat hypothalamus and preoptic area, estrogen desensitizes \( \beta \)-adrenergic receptor stimulation of adenylate cyclase without modifying G protein stimulation of the enzyme. In the present study, we examined whether estrogen modulates receptor function through changes in either receptor density or binding affinity. We examined receptor binding affinity and density using the high specific activity \( ^{3} \)\(^{2} \)iodocyanopindolol (\( ^{3} \)\(^{2} \)ICYP) in membranes prepared from combined hypothalamus-preoptic area of ovariectomized (OVX) and OVX, estrogen-primed rats. OVX rats received oil vehicle or 2 \( \mu \)g of estradiol benzoate 48 and 24 h prior to sacrifice. Specific binding of \( ^{3} \)\(^{2} \)ICYP in hypothalamus-preoptic area membranes was not different from controls. Experimentation carried out in cortical membranes also showed no noticeable difference in affinity of estrogen on antagonist binding affinity or receptor density. These data suggest that estrogen-dependent uncoupling of \( \beta \)-adrenergic receptor from adenylate cyclase is not contingent on hormonal modulation of receptor density or antagonist binding affinity. Agonist affinity studies are currently underway to determine if estrogen regulates the coupling of \( \beta \)-adrenergic receptors to adenylate cyclase by modulation of agonist binding.


The aminosteroid, U-73122 (1-(6-[[17b-3-methoxyestr-1,3(10)-trien-17-y]amino]ethyl]-1-hydroxy-2,5-dione) reportedly inhibits muscarinic receptor sequestration and polyphosphoinositide (PPI) hydrolysis in SK-N-SH cells. Like CGMP, the muscarinic receptor, the neurotensin receptor couples to cyclic guanosine 3',5'-monophosphate (cGMP) synthesis and PPI hydrolysis. For this study, we examined the effects of U-73122 on neurotensin or muscarinic receptor mediated CGMP formation and PPI hydrolysis in murine neuroblastoma clone NIE-115. Both CGMP formation and PPI hydrolysis stimulated with 10 nM neurotensin or 1 mM carbachol (a muscarinic receptor agonist) were inhibited dose dependently after incubation with U-73122 (dissolved in DMSO, 1% final) at 37°C for 15 min. Maximum inhibition occurred with 10 \( \mu \)M U-73122. We showed before that 10 \( \mu \)M U-73122 inhibits neurotensin receptor down-regulation in NIE-115 cells. Molecular mechanisms underlying these phenomena are unknown. However, our results suggest that U-73122 inhibits the down-regulation, CGMP formation, and PPI hydrolysis in many other receptors that couple with these responses (Supported by Mayo Foundation and USPHS grant MH27692).


The sympathoadrenal system is characterized by the expression of neuropeptides (CA) and several neurotransmitters. Electrical activity, elicited by the presynaptic fibers, influences the survival, maintenance and phenoic expression of the sympathetic neurons. We are using acute adrenal gland and sympathetic neural cultures from 11 day chick embryos to study the effect of depolarization on CA and neuropeptide expression (enkephalins, neuropeptide Y (NPY) and somatostatin (SS) expression). Histoneological studies show, that, as occurs in vivo, CA, ENK, NPY, and SS-like immunoreactivity is expressed by sympathoadrenal neurons. Radioimmunoassay for ENK and NPY show that their expression is enhanced in both cell cultures upon depolarization by 30 mM K+ or veratridine (10 mM). SS levels, on the contrary, are lowered or do not change. These changes may be mimicked by the Bay K 8644 (1 \( \mu \)M) but not by the adenylate cyclase activator forskolin (10 \( \mu \)M), suggesting the involvement of Ca?. However, no change is observed in somatostatin levels in sympathetic neuronal cultures. Our results show that electrical activity modulates the expression of neuropeptides and CA in the avian sympathoadrenal system and that the alteration of a specific effect might occur in vivo.


Calcitonin gene-related peptide (CGRP) is produced by the alternative preprocursors of the calcitonin/CGRP gene primary transcript. CGRP is a potent vasodilatory neuropeptide and has been implicated in regulation of cardiovascular function. Neuropeptide expression in dorsal root ganglia (DRG) neurons has been shown to be modified by centrally administered CGRP or CGRP-like agonists. We have therefore begun to use primary cultures of dorsal root ganglia (DRG) neurons to identify the factors that modulate CGRP mRNA levels. We find that this is the site of neuronal cell bodies known to produce abundant CGRP levels and to send axons peripherally to peripheral tissue and centrally to spinal cord. It has been previously documented that isolated DRG neurons produce CGRP and that many of both mRNA and protein are positively regulated by NGF. Using Northern blot analysis and immunocytochemical staining we have confirmed that DRG neurons contain CGRP expressing cells. Preliminary results indicate that treatment (20h) of isolated neurons with either forskolin (10\( \mu \)M) or phorbol 12-myristate 13-acetate (PMA; 20\( \mu \)M) in absence of NGF also increase CGRP mRNA levels. These results suggest that regulatory agents which act through protein kinase A and protein kinase C signal transduction pathways may modulate neural CGRP expression.

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422.26 G PROTEIN INVOLVEMENT IN COCAINE SENSITIZATION. C.D. Striplin and P.W. Kalivas, Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Behavioral sensitization is associated with a significant increase in dopamine neurotransmission in the nucleus accumbens (NA). While the neurochemical initiation of behavioral sensitization is thought to occur in the ventral tegmental area (VTA), a role for G proteins has been hypothesized since daily cocaine (30 mg/kg X 10 days) has been shown to decrease the levels of Gi and Go proteins. Also five daily injections of 15 or 30 mg/kg cocaine was shown to decrease the level of V12 pertussis toxin ribosylation 1-2 hours after the last injection and a significant correlation has been found between the level of behavioral sensitization observed at 15 mg/kg and pertussis toxin ribosylation in the VTA. To determine if the Gi protein changes were translated into lasting, rats were sensitized to cocaine for 5 days and then given a final challenge of cocaine 14 days later. Rats were killed 1 hour after the final injection and G proteins were analyzed in the VTA, NA, substantia nigra, striatum and prefrontal cortex by Western blot analysis. The levels of Go, Gi, and Gs did not show any significant change in any of the brain areas tested. However, a significant decrease in GiI levels was observed in the NA in chronic cocaine treated animals given an acute cocaine challenge (85.5%) and chronic cocaine treated animals given an acute saline challenge (89.2%). These data indicate that G proteins in the VTA may play a role in the initiation of sensitization while long-term sensitization is associated with G protein changes in the NA.

423.1 REVERSIBLE DEPLETIONS OF 5-HYDROXYTRYPTAMINE IN THE NEO- 

5-hydroxytryptamine (5-HT) systems subserve both neuro-a-
transmitter and neurotrophic functions in the rat. Recent
data showed that 5-HT can regulate the density of gluco-
corticoid receptors (GCR) in fetal hippocampal cell
cultures. Elevated GCR levels are associated with
enhanced negative-feedback control of the hypothalamic-
–pituitary–adrenal axis (HPA). We have examined the effects of
perinatal 5-HT depletions on stress responses of the adult
rat in order to assess the trophic action of 5-HT on HPA function.

Neonatal rats were injected with PCPA or vehicle (VEH)
on alternate days, up to day 8. Plasma corticosterone (B) was measured in response to 20 min restraint stress in
adulthood.

Although basal levels of B were the same for both
groups, PCPA-treated rats hypersecreted B upon termination
of stress and at 20 + 60 min post-stress. By 120 min post-
stress both groups had returned to basal levels.

These results indicate that 5-HT is required for HPA function, presumably by some trophic action. We are
currently investigating the specific brain locations and
nature of this regulation.

423.2 CHANGES IN PLASMA ACTH, CORTICOSTERONE, CBG, AND HIPPOCAMPAL GLUCOCORTICOID RECEPTOR OCCUPANCY IN RAT PUPS FOLLOWING MATERNL SEPARATION AND/OR EITHER STRESS Shakti Sharma*, Y. Vyas, S. LaRocco and M.J. Mosey, Douglas Hos, Res. Ctr., Depts. of Psychiatry, and Neurology and Neurosurgery, McGill Univ, Montreal, Canada H4H 1R3.

Plasma ACTH and corticosterone (B) responses to stress are often reduced in the neonatal rat. However, plasma corticosteroid-binding globulin (CBG) levels of the neonate are substantially decreased, which might obscure the biological significance of existing glucocorticoid levels. We examined this question by estimating hippocampal glucocorticoid receptor (GR) occupancy in Day 6, Day 15, and adult animals under basal and stressed conditions. The results showed that: 1) Plasma ACTH levels were elevated in Day 6 animals in response to acute stress and maternal separation, and maternal separation + ether, however, ACTH responses were substantially lower than in Day 15 or adult animals. 2) Plasma total B levels followed a similar pattern, most noteworthy was the potent glucocorticoid response in Day 15 animals to the combination of maternal separation + ether. 3) Plasma CBG levels in Day 6 animals were extremely low (<3% adult values) by Day 15 CBG levels were about 25% of adult levels. Interestingly, maternal separation was associated with a substantial decrease in plasma CBG levels. 4) Hippocampal GR occupancy was similar at all ages under both basal and stress conditions. The only notable exception occurred during maternal separation in Day 15 animals, where hippocampal GR occupancy was higher than that observed at any time in any other group tested. The results are likely related to the decrease in plasma CBG that occurs following separation of Day 15 pups from the dam. Thus, despite the differences in plasma CBG levels, GR occupancy was generally comparable across all ages either under basal conditions, or following stress. These receptor data underscore the importance of developmental changes in plasma CBG levels.
423.3 DEVELOPMENTAL MANIPULATIONS DIFFERENTIALLY AFFECT HPA AXIS OF MALE AND FEMALE RATS. C. M. McCormick*, J. W. Smythe, S. Sharma, & M. J. Meaney, McGill University, Dept. of Psychiatry, Douglas Hospital Research Center, Montreal, CANADA H4H 1R3.

Hypothalamic-pituitary-adrenal (HPA) function was investigated in adult animals in relation to both prenatal stress (PS: 20 min restraint of mothers on days 15-19 post-conception) and neonatal handling (H: separation from mothers for 15 min daily during first 10 days of life). Blood samples were drawn from catheters prior, during, and following 20 min restraint. PS females showed elevated levels of ACTH and corticosterone (B) during and following stress compared to NS females. No marked differences in B, ACTH, or corticosterone binding globulin (CBG) were observed between PS and NS males. Despite similar basal levels of B, PS females showed elevated levels of CBG compared to NS females, which may account for their increased HPA response to stress. PS was associated with increased glucocorticoid receptor (GR) levels in the frontal cortex (FC) in females, decreased levels in FC of males, and increased levels in septum (3Pt) of males. PS was not associated with GR levels in hippocampus (HPc), hypothalamus (HYPO), and amygdala (AMG). In contrast, neonatal handling (H) was associated with a reduced HPA response to stress in both males and females. H animals showed increased levels of GR in HPC (both sexes) and FC (males only), and decreased GR levels in 3Pt (males only) compared to non-H animals. H was not associated with GR levels in HYPO or AMG. Across experimental groups, when sex differences were observed, they were in the direction of higher GR levels in females than in males.

The results reflect sex differences in the effects of early environmental events on (1) HPA response to stress, and (2) GR levels in brain regions implicated in HPA regulation, and (3) plasma levels of CBG.

423.5 PLASMA CORTICOSTEROIRE IS SIGNIFICANTLY INCREASED BY COLD STRESS IN THREE-DAY-OLD NEONATAL RATS. S. J. Yi-L. Schutz, T. Z. Haram, Div. of Neurology, Children's Hospital Los Angeles, Los Angeles, CA 90027.

The hypothalamic-pituitary-adrenal axis underlies the hormonal stress response. The responsiveness of the neonatal hypothalamic-pituitary-adrenal axis to stress has been thought to be impaired ("stress hyporesponsive period"). We studied the response of neonatal rats to cold stress by measuring plasma corticosterone levels.

Rats aged 3 to 17 days were placed in compartmentalized plastic cages in a cold room (4°C) for 20 to 60 min. Trunk blood was collected 5 min., 20 min, 1 h, 4 hours and 23 hours after the onset of cold stress for plasma corticosterone measurement by RIA. Littermate control rats were kept under "stress-free" conditions.

Animals exposed to cold stress showed a marked increase in plasma corticosterone starting on the third postnatal day, compared to non-stressed controls. We are currently studying the response of plasma ACTH and CRH mRNA abundance in the paraventricular nucleus to cold stress during the first postnatal week.
PLASTICITY OF $\beta$-ADRENERGIC RECEPTORS ON SPLENCYCOTES FROM YOUNG AND OLD RATS FOLLOWING CHRONIC SYMPATHETICOMYSIS. S.M. Bremner, D.L. Bellinger, K.S. Maddox, A. Toty, J. Housel, S.F. Felten and D.L. Felten. Department of Neurology, University of North Carolina School of Medicine, Chapel Hill, NC 27599.

$\beta$-adrenergic (BAR) density on spleenocytes was examined in 3- and 21-month-old (M) F344 rats between 1 and 5 days (D) after chemical sympathectomy (SymXp) with 6-hydroxydopamine. In spleenocytes from 3-M SymXp rats, $\beta$-adrenergic receptor density was not significantly different from nontreated or vehicle-treated controls at D1-3 post-treatment, but progressively increased through D10, and then declined from D10 through D56. BAR density at D56 was significantly higher compared with both controls, respectively. Vehicle-treated 3- and 21-M animals possessed a slightly higher density of BAR on spleenocytes than did nontreated age-matched controls. Spleenocytes from 21-M SymXp rats, a progressive decline in BAR density was observed between D1 and D3, followed by a progressive rise in BAR density through D56. At D56 the density of BAR on spleenocytes from 21-M rats was not significantly different from vehicle-treated controls. These findings indicate that regulation of BAR density on spleenocytes following acute denervation is impaired with age; upregulation of BAR in response to denervation was not observed, only a slow progressive increase in the receptor density that closely parallels the reinervation of the spleen in 21-M rats. The initial decline in BAR following SymXp was followed by a slow decline in the ability to metabolize norepinephrine released from damaged sympathetic nerves. Supported by 1 R29 MH47783, R37 MH42076, and Markley Foundation Center. Award.

PLASTICITY OF NORADENERGIC NERVES IN SPLEENS FROM AGED F344 RATS FOLLOWING CHEMICAL SYMPATHETOMY. K.S. Maddox, D.L. Bellinger, A. Toty, J. Housel, N.L. Costello, C. Richardson, S.F. Felten, and D.L. Felten. Department of Neurology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

The time course and pattern of noradrenergic (NA) nerve ingrowth into the spleen following sciotic sympathectomy with 6-hydroxydopamine (6-OHDA) was examined in 3- and 21-month (M)-old male F344 rats using glycinic acid fluorescence histochemistry and biochemical measurement of norepinephrine (NE). NE and 6-OHDA from 3-M-old rats were localized preferentially along the splenic artery and entered the hilum (1-5 days, D1-5), extended into the hilus region (D5-D10), and then proceeded into regions distal from the hilus (D10-D56), suggesting orderly ingrowth from the hilum to distal regions. NA nerves innervated the same compartments seen in vehicle controls, but nerve fiber density in these compartments differed based on distance from the hilum and time after denervation. By D56 post-lesion, regions distal from the hilum had fewer NA nerves than vehicle controls, suggesting that reinnervation does not restore nerve density identical to that of vehicle controls. In contrast, spleen NE concentration at D56 post-lesion returned to vehicle control values, suggesting that functional reinnervation of NA nerves in the spleen may involve metabolic and receptor compensation for the lack of complete fiber reingrowth into regions of white pulp distal from the hilum. NA nerves from 21-M-old rats were involved in a different ingrowth pattern into the spleen following denervation; however, the initial ingrowth was delayed until D15 after the last dose, ingrowth occurred over a slower time course, and the density of NA nerves returning to the splenic pulp was reduced at D56 compared with all other young and old treatment groups. Spleen NE levels at D56 post-lesion also were significantly lower than in 21-M-old vehicle controls, suggesting that plasticity of NA innervation is compromised with age. Supported by 1 R29 MH47783, R37 MH42076, and Markley Foundation Center. Award.

PAVLOVIAN CONDITIONING OF MORPHINE-INDUCED IMMUNE ALTERATIONS: EVIDENCE FOR OPIOID RECEPTOR INVOLVEMENT. Mary E. Cournoyer*, Linda A. Dykstra, and Donald T. Lysle. Departments of Psychology and Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3270.

Our prior work has shown that morphine’s immunomodulatory effects can become conditioned to environmental stimuli that predict drug administration. These alterations include conditioned changes in natural killer cell activity, interleukin-2 production, and mitogen-stimulated lymphocyte proliferation. The present studies were aimed at determining the involvement of opioid receptor activity in the establishment and expression of morphine-induced conditioned immune alterations. During the training phase, Lewis rats received two conditioning treatments each including a subcutaneous injection of 15 mg/kg morphine sulfate was paired with exposure to a distinctive object. On the test day, animals were reexposed to the distinctive object alone prior to sacrifice. Saline or naloxone (3, 10, 30, 100, 400 mg/kg) was administered either prior to training or to test. Administration of naloxone prior to training resulted in attenuation of the conditioned immune alterations, whereas naloxone administration prior to testing had no effect. Taken together, these studies show that opioid receptor activity is involved in the establishment, but not the expression, of conditioned morphine-induced immune alterations. (Supported by PHS grants DA00749, DA07244 and MH46284.)

PRESENCE AND AVAILABILITY OF VIP IN PRIMARY AND SECONDARY LYMPHOID ORGANS. D.L. Bellinger, D.J. Eantwort, M. Gallagher, and D.L. Felten. Department of Neurobiology & Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

A large body of pharmacological and immunological data indicate that vasoactive intestinal peptide (VIP) acts as a neurotransmitter with cells of the immune system as targets. VIP receptor (VIPR) mRNA is present in human T and B lymphocytes. In functional studies, VIP inhibits mitogen-induced proliferation of lymphocytes from human T cell lines and human peripheral B lymphocytes. Although VIP receptors in T lymphocytes mediate their mitogenic activity, VIP receptors in B lymphocytes mediate their proliferative activity. The initial rise in BAR density suggests an upregulation of receptors in response to denervation of the spleen. The subsequent progressive decline closely parallels the time course of appearance of BAR on nerve fibers in the spleen. BAR density on spleenocytes from nontreated and vehicle-treated 21-M rats was not significantly higher with that seen in 3-M rats (approximately 90-900 and 475-525, respectively). Vehicle-treated 3- and 21-M animals possessed a slightly higher density of BAR on spleenocytes than did nontreated age-matched controls. Spleenocytes from 21-M SymXp rats, a progressive decline in BAR density was observed between D1 and D3, followed by a progressive rise in BAR density through D56. At D56 the density of BAR on spleenocytes from 21-M rats was not significantly different from vehicle-treated controls. These findings indicate that regulation of BAR density on spleenocytes following acute denervation is impaired with age; upregulation of BAR in response to denervation was not observed, only a slow progressive increase in the receptor density that closely parallels the reinervation of the spleen in 21-M rats. The initial decline in BAR following SymXp was followed by a slow decline in the ability to metabolize norepinephrine released from damaged sympathetic nerves. Supported by 1 R29 MH47783, R37 MH42076, and Markley Foundation Center. Award.

MORPHINE-INDUCED ALTERATIONS OF IMMUNE STATUS: EVIDENCE FOR $\beta$-ADRENERGIC RECEPTOR INVOLVEMENT. Karamarie Fecho,* Donald T. Lysle and Linda A. Dykstra. Department of Psychology & Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

There is evidence suggesting that morphine’s immunomodulatory effects are mediated through the central nervous system. For example, the systemic administration of morphine, but not N-methylmorphine, has been found to produce a naloxone-reversable suppression of splenic NK cell activity. However, little is known about how the activation of opiate receptors by morphine translates into peripheral immune alterations. The purpose of the present experiments was to investigate at the level of the $\beta$-adrenergic system as one possible mediator of morphine’s effects. Prior to a subcutaneous (s.c.) injection of either 15 mg/kg morphine or saline, male Lewis rats (N = 180) were administered either the nonspecific $\beta$-adrenergic receptor antagonist nadolol, the selective $\beta$-adrenergic receptor antagonist atenolol or the selective $\beta$-adrenergic receptor antagonist ICI-118,551 in doses of 0, 0.125, 0.5, 2.0 or 8.0 mg/kg, s.c. All three antagonists dose-dependently attenuated the suppressive effects of morphine on the proliferative responses of splenic lymphocytes to Con-A, PHA, LPS and Iono/PMA. In contrast, none of the antagonists exhibited any effect on the morphine-induced suppression of the proliferative responses of blood lymphocytes to Con-A or PHA; likewise, there was no antagonism of the suppression of splenic NK cell activity produced by morphine. Although the immunosuppressive effects of morphine appear to involve multiple mechanisms, this data clearly implicate the involvement of morphine’s effects.

MODULATION OF ANTIBODY PRODUCTION AND ANTIGEN-INDUCED PROLIFERATION BY A CONDITIONED AVERSIVE STIMULUS. Elizabeth H. Bennett, Lynn Perez, Maureen E. Bronson* and Donald T. Lysle. Department of Psychology & Curriculum in Neurobiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599.

Our research program is designed to characterize the immunomodulatory effect of a conditioned aversive stimulus (CS) in rats. The present study examined the effect of presentation of the CS on the proliferative response of lymphocytes to the T-dependent antigen, keyhole limpet hemocyanin (KLH) and the production of specific antibody to KLH. The results showed that presentations of the CS on days 0, 2, and 4 following immunization had no effect on the proliferative response to KLH or the production of KLH-specific antibody. Furthermore, presentation of the CS on days 0 and 2 had no significant effect on antibody production. In contrast, presentations of CS on days 10, 12, and 14 following immunization induced a significant suppression of the KLH-induced proliferative response of lymph node lymphocytes, but not splenic lymphocytes, and a reduction in serum antibody levels to KLH. These findings indicate that a conditioned aversive stimulus can modulate the immune system, and the effect is dependent upon the temporal relationship between antigen administration and presentation of the CS. (Supported by MH46284.)
CHARACTERIZATION OF OPIOID RECEPTOR INVOLVEMENT IN PAVLOVIAN CONDITIONED IMMUNE ALTERATIONS. Lynn Perry and Donald T. Lysle*. Department of Psychology & Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

Previous work from our laboratory has shown that naltrindone can antagonize alterations in immune status induced by presentation of a Pavlovian conditioned stimulus (CS). The present studies were designed to further this investigation by determining the specific opioid receptor subtype involved in conditioned immune alterations. 8-1 naltrindone (8-FNA), a highly selective a opioid-receptor antagonist, was administered (0, 5.0, and 25.0 mg/kg, i.p.) 24 hours prior to the CS. Alterations of immune status were determined by in-vitro assessment of natural killer cell (NK) activity, lymphocyte proliferation induced by Con-A, PHA, LPS, and ionomycin/PMA, and production of interleukin-2 (IL-2). 8-FNA antagonized the CS-induced suppression of NK activity, but did not attenuate the suppression of lymphocyte proliferation or IL-2 production. In a subsequent study, naltrindone, a selective 6 opioid-receptor antagonist, was administered (0, 0.1, 1.0, 10.0, and 30.0 mg/kg, i.c.) prior to exposure to the CS. The results showed that naltrindone had no effect on the CS-induced alteration of immune status. To further characterize opioid receptor involvement in conditioned immune alterations, additional studies will investigate the role of kappa opioid receptors using non-biarylpropionamides, a k-selective antagonist. Collectively, the results of these studies provide an extensive analysis of the involvement of opioid receptor subtypes in conditioned immune alterations. (Supported by MH46284.)

424.7

424.9


Administration of adrenal steroids is classically associated with increases in neutrophils and decreases in lymphocytes and monocytes in the peripheral blood. Although immune cells possess two types of adrenal steroid receptors, type I (mineralocorticoid) and type II (glucocorticoid), it is unknown which receptor subtype is involved in the adrenal steroid effects. To examine this issue, male rats were treated with selective receptor agonists for 7 days via osmotic minipumps and the number and percentage of immune cells in the peripheral blood was determined. For each receptor agonist (aldosterone for the type I receptor, RU28362 for the type II receptor), five groups of rats (300g) were studied: no hormone treated controls (ADX); 7-day ADX; 7-day ADX+1mg/kg; 7-day ADX+4mg/kg and 7-day ADX+10mg/kg. Whereas ADX alone had no effect on the total white blood cell (WBC) count, both aldosterone and RU28362 resulted in a significantly decreased WBC count whereas aldosterone decreased the number of neutrophils, lymphocytes, and monocytes; RU28362 decreased lymphocytes and monocytes but was associated with a significant increase in neutrophils. As for lymphocyte subtypes, RU28362 had the greatest inhibitory effect on T helper cells and B cells, while aldosterone was associated primarily with decreases in T cells and NK cells. These results suggest that the two adrenal steroid receptor agonists have different effects on peripheral blood immune cells in the rat. Since selective activation of adrenal steroid receptor subtypes can occur under physiologic conditions, these differences allow for complex and varied effects of adrenal steroids on immune cells under basal hormone conditions and following stress. (Supported by MH06080, MH47674)

424.10

EFFECTS OF ENDOXTON ON THE INDUCTION OF C-FOS PROTEIN IN THE BRAIN. PLASMA LEVELS OF CORTICOSTERONE, AND NOREPINEPHRINE AND VIP LEVEL IN THE BRAIN OF THE RAT. C. Y. Tse†, W. Wam, L. J. M. Serrulli*, A. J. Greenberg and D. M. Nance. Department of Psychology and Institute of Cell Biology, University of Manitoba, Winnipeg, MB, R3E 0W3, Canada and *Oncogene Sci., Inc., Uniondale, NY 11553. Lipopolysaccharides (LPS), an endotoxin associated with gram-negative bacteria, is a potent activator of the immune system. We have tested the effects of LPS infusions of LPS (10 ng) i.g. of LPS on the induction of the protocin receptor protein in the brain in the brain as well as plasma levels of corticosterone and spastic concentrations of norepinephrine (NE) and VIP. The dose was chosen because of its reported cross-reactivity with a polyclonal antibody to c-fos (1,000 Oncogene Sci.) and the PAP technique. At 3 hr post-LPS infusion of LPS, we observed numerous labeled neurons focused in the paraventricular nucleus (PVN) of the hypothalamus and the A2 region of the brain stem. Corticosterone levels as well as spinal levels and VIP levels were all elevated 3 hr post-LPS. LPS, relative to Ringer's solution-place rats. In addition to the c-fos labeled neurons, we also assessed the course for the induction of c-fos labeled neurons in the brain following IP injections of LPS as well as establish a dose response curve for c-fos labeling in the PVN vs IP dose of LPS. Labelled cells were examined in the PVN 1 hr following IP injection (100 ng), increased further at 2 and 3 hr post-injection and then returned to control levels at later intervals. The dose response curve for IP LPS vs the number of c-fos labeled neurons in the PVN showed a few labeled cells detectable at a dose of 4.0 ng, but the number and staining intensity of labeled neurons increased up to a dose of 100 ng, with no further increase in the number of labeled neurons at higher doses. In contrast to IP injections, we observed additional labeled cells in the suprachiasmatic nucleus (SON), arcuate nucleus and the A1 regions of the brain stem at IP doses of 40 ng higher. These results indicate selective and differential effect of central and peripheral LPS on the induction of c-fos protein in hypothalamic and brain stem nuclei. The concurrent changes in corticosterone level and spastic neurotransmitter levels produced by IP LPS suggest that the endocrine and autonomic nervous systems are primary targets for these activational effects of endotoxin. (Supported by MRC, NSERC and HSFRC.)

424.11


We have previously demonstrated that estrogen increases the affinity of thymic oxytocin (OXT) receptors. This study tested whether an E cell lipopolysaccharide (LPS) challenge would alter OXT immunoreactive levels or thymic OXT receptors in ovariectomized (OVXed) rats given 5 μg estradiol benzoate (EB) once daily for three days or on day 4. The fourth day rats received iv infusions of 150 μg/kg LPS or saline vehicle 90 min, before decapitation. LPS resulted in a significant increase in blood OXT levels only in estradiol-pretreated rats. The thymus, MPOA, PVN, and the adrenals showed no significant changes in OXT levels with these treatments, although OXT levels in the PVN were correlated with blood levels, while MPOA levels correlated with adrenal levels. Computerized analysis of total brain OXT binding experiments in which 0.2 to 200 nM OXT were incubated with 0.2 μM [125I]OXT-OA indicated that thymus from oil-saline treated OVXed matched a two-site model with only high affinity (K: 0.39 ± 0.08) and low-affinity (K: 9.3 ± 0.3) OXT binding sites. The EB-saline, oil-LPS and EB-LPS treated animals demonstrated one-site models with only high affinity models (Ks were 0.39, 0.42 and 0.42 respectively). In vitro experiments 100 nM OXT or 40 μg LPS added to homogenates of thymus from OVXed oil-saline vehicle treated animals also resulted in the loss of the low-affinity sites (one-site model with K: 0.46 for both). OXT release after an immune challenge is estrogen-dependent while LPS directly reduces the thymus by eliminating a population of low-affinity OXT receptors. (Supported by MH46808.)

424.12


Amino acids capable of producing excitation and subsequent autonomic arousal lesions are used as neuroendocrine research tools. Studies of brain regulation of PRL and adrenal hormone release after systemic N-Methyl-D-L-Aspartic Acid (NMA) administration have demonstrated differing thresholds for the modulation of individual pituitary hormones. The prominent role of PRL and cort in the modulation of immune function coupled with the possibility that these hormones may also have a differential release in response to PRL and cort in the following experiments. Male rats received intrasutural blood samples (bs. Oxy, AD and AM) prior to bilateral injections of either artificial CSF (aCSF) or NMA (0.453 mg/kg in 0.1 in 0.1 ml) at 2 min the anterior hypothalamic region (AHA). At 3 and 72 post NMA rats received 1 or 1.5 mg/100g. E cell endotoxin (Endotox) or saline per carcine at bs at 8 hr (AM) and again at 0.2, 1.3, 1.6 and 24 hr post endotoxin. Cort and PRL were assayed 2 min the treatment of control rats resulted in 8X max increase (from baseline) in plasma Cort at 0.5 hr and a 5X max increase in Prol at 1.5 hr which returned to baseline at 3 hr. Injection of either aCSF alone or NMA resulted in a 10X increase in Cort and NMA into AHA 72 postinjection fail prevent cort release in controls. However, both injection of aCSF alone or with NMA resulted in a respective, progressive production in PRL release after Injection of either of aCSF alone or NMA into AHA 72 postinjection fail prevent cso release. Summary increase in Prol and Cort release after Endo occurs with the change the latter occurring sooner and lasting longer. Cort release after endotox is not hampered after progressive damage to AHA, whereas Prol release was blocked by small (0.1mm) lesions. (Supported by MH46808.)

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342.14

ENDOTOXIN CHALLENGE IN THREE-DAY-OLD RAT PUPS: HYPOTHALAMIC-PITUITARY-ADRENAL ACTIVATION AND DEVELOPMENTAL CONSEQUENCES
N. Shanks* & M.J. Meaney, Douglas Harvey Research Center, Dept. of Psychiatry, McGill University, Montreal, Quebec, H4H 1R3 CANADA

Antigenic challenge with endotoxin (ENDO) is known to activate the hypothalamic-pituitary-adrenal axis (HPA) in adult animals. Recent data have implicated IL-1β and corticotropin releasing hormone (CRH) as potential mechanisms responsible for HPA activation during the acute phase response to antigenic challenge. However, relatively little is known about HPA and immune interactions during development and whether neonatal immune challenge alters endocrine functioning as an adult. A series of studies were performed assessing the HPA response to salmonella enteritidis endotoxin challenge in three-day-old rat pups. It was observed that END0 administration at doses of 33μg/kg (ip) provoked marked elevations in both plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT), while only slightly altering blood glucose levels. HPA activation peaked between 3 to 4 hours following END0 administration in both male and female rat pups. However, a marked sex difference was evident with respect to both the dynamics and magnitude of the HPA response. The magnitude of both the CORT and ACTH responses to END0 were both greater and occurred earlier in female rat pups relative to males, however, hormone levels returned to basal values within 24 hrs in both sexes. In addition, endotoxin-induced CRH content decreased 2.4 times following END0 in both male and female rat pups, suggesting that the activation of the HPA axis was mediated by END0 stimulated CRH release. Studies are presently underway to investigate interactions between CRH and IL-1β in the neonate, and assess the long-term consequences END0 challenge during development on HPA function and its relation to gender.

342.15

BRAIN AND LYMPHOCYTES BETA-ENDORPHIN AND SUBSTANCE P IN EXPERIMENTAL ALLERGIC ENCEPHALITIS (EAE).
J. Velicic, P. Sacerdote*, G. Monastir, A. E. Frangini Dep. Pharmacology, School of Medicine, University of Milano, 20133, Milano, Italy and Fidia, 53031, Abano Terme, Italy.

Evidence is rapidly cumulating that neurotransmitters participate in the modulation of immune responses acting both in the central nervous system, and with autocrine, paracrine or endocrine mechanisms. We measured beta-endorphin (BE) and substance P (SP) concentrations in discrete brain regions cell lines and brain tissue (ILC of EAE sensitive) and Brown Norway (EAE fully resistant) rats before and every other day after treatment with immunoprotective spinal cord homogenates (ICP) or bovine serum albumin (BSA), together with Freund adjuvant and Bordetella Pertussis. At the same time intervals, also tumor necrosis factor (TNF) released from splenocytes was measured. In Lewis rat BE increased after GP or BSA in all the brain areas studied, peaked on days 12/14 and declined by day 21. Increases were significantly higher after GP than BSA. A minor increase with the same pattern was also present in Brown Norway rats. SP concentrations showed a reverse pattern, with nadir on day 12/14. In LC, BE concentrations increased on day 6 after immunization with GP or BSA and returned to normal values thereafter. TNF release from splenocytes increased significantly on days 10 and 12 only in Lewis rats. No correlation was found with clinical scores of the disease, but chronic treatment with the opiate receptor antagonist naltrexone starting on the day of immunization aggravated the clinical scores. The data presented are consistent with an inhibitory role for BE on the immune system and on the development of EAE.

342.17

MORPHOLOGICAL CHANGES IN THE HIPPOCAMPUS OF THE CONGENITALLY ATHEMIC (nu/nu) MOUSE.
K. C. French, O. G. Flugel, J.A. Reyes and M.C. Melnick*. Department of Endocrinology, University of California, Berkeley, CA 94720

Preliminary data in our laboratory show morphological differences in the hippocampus of 120-day-old, female homozygous nude (nu/nu) and balb/c (Balb/c/Balb/c) mice. Measurements of thionin stained sections of the hippocampi corresponding to CA1 and CA2+CA4 were taken from nude (N=13) and Balb/c (N=9) mice.

Our results indicate that the hippocampus in both the right and left hemispheres of the brain was significantly thiner in the nude than in balb/c mice. In the right hemisphere, the CA1 hippocampal region was thinner by 5.9% (p<0.05) and the CA2+CA4 region thinner by 17.6% (p<0.01) in the nude compared to the Balb/c mouse.

In the left hemisphere, the CA1 region was thinner by 5.1% (p=0.02) and the CA2+CA4 region thinner by 11.8% (p<0.001) in the nude compared to the balb/c mouse.

We are currently investigating central cortical and hippocampal morphology in female nude and Balb/c mice with the addition of the hormone regimens (nu/nubalb/c) as a control. Our purpose is to gain insight on regulatory hormones or factors that may be involved in mediating the deficiencies observed in brain morphology.

We are approaching this problem by grafting thymic and/or pituitary tissue in 5 week-old, female homozygous nude mice. Using radioimmunoassay, we will examine levels of prolactin, the primary hormone released from extrapituitary graft, corticotropin, and ACTH binding (T4 and T3). In addition, we will observe changes in CD4 and CD8 lymphocyte population using fluorescence-activated cell sorter (FACS) in the different experimental groups.

342.18


Enhancement of peripheral catecholamine release from nerve terminals and the adrenal medulla has been suggested to occur during septic shock using whole-animal experiments. Since mononuclear cytokine expression is an important response to sepsis, the present study tested possible neuroendocrine-immune-interactions in vitro by examining the effect of mitogen-stimulated mononuclear cells on catecholamine secretion from chromaffin cells. Chromaffin cells, isolated from rat brains with collagenase digestion, were maintained in 2 cm² wells 4-5 days with DMEM and F12 with 10% FCS in 5% CO2 at 37°C (0.6 x 10⁶ cells/well, 1.0 ml). Splenocytes were isolated from bovine spleen and cultured in RPMI with 10% FCS and stimulated with the CSF (1:10) and stimulated with 0.5% phytohemagglutinin (PHA). After 24 hrs. PHA-conditioned media (PHA+) was isolated and frozen. In secretion experiments chromaffin cell growth media was replaced with conditioned media at the time 0. In both the CSF and PHA stimulated, secretion was measured at variable times. After removal of PHA+ or control media, cells underwent mitogenic stimulation (3 μg Dimethylsulfoxipidermin (DMSO) for 10 min. E release was measured and E release expressed as a percentage of E released in initial E control. After 90 min with PHA+ media, E secretion increased to 19.1±0.5% of total (P<0.05 vs other treatments) compared to 5.2±0.3% for PHA- extract, 3.3±0.4% for media alone, and 7.4±0.5% for media alone (N=4 chromaffin cell preparations). E secretion was 25% by 210 min with PHA+ E release was dose-dependent. Nontoxic stimulation resulted in 0.6±0.6% release in E after 90 min with PHA+ (P<0.05 vs all other treatments) compared to 3.1±0.3% for PHA-, 2.7±0.2% for media + PHA and 2.4±0.4% for media alone (N=4 chromaffin cell preparations). Enhanced mitogenic secretion was also dose-dependent. The results support the concept that sympathetic response to sepsis is in part due to peripheral modulation of catecholamine secretion. (Supported by the Bane Foundation)
424.20

**EFFECT OF IONIZING RADIATION ON RAT'S HIPPOCAMPAL NORADRENALINE RELEASE. S. B. Kandasamy*, S. Blakely, T. K. Dalton and A. H. Harris. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20880-5145.**

The purpose of this study was to examine the effect of ionizing radiation on hippocampal noradrenaline (NE) release in vitro, stimulated by KCl (0.5 h, 24 h, 47 h) and sham exposure. Rats were irradiated using a 60Co source. The levels of NE were measured by HPLC coupled to electrochemical detection. There was no significant change in NE release between irradiated and non-irradiated rats when the hippocampal NE was determined after 0.5 h irradiation (5-30 Gy at 10 Gy/min) or 47 h after sham irradiation/sham exposure. However, there was a significant decrease in NE release 48 h, and 72 h after irradiation (10 Gy at 10 Gy/min). Pretreating rats with 3-10 μg/kg of recombinant human interleukin-1β (rhIL-1β) or 3-10 μg/kg of corticotropin releasing factor (CRF) administered IP 1 h before sham irradiation enhanced hippocampal NE release and the same treatment prevented the radiation-induced decrease in NE release 8 h after irradiation. These results suggest that ionizing radiation induces decrease in hippocampal NE release 24 h, 48 h, and 72 h after exposure and pretreatment with rhIL-1β or CRF prevents the decrease.

**NEURAL-IMMUNE INTERACTIONS: INTERLEUKIN-1**

425.1

**BRAIN INTERLEUKIN-1 (IL-1) ENHANCES NOCICEPTION IN THE RAT. T. Hoet*, T. Oka and S. Aou, Dept. of Physiology, Kyushu University Faculty of Med., Fukuoka, 812, Japan.**

IL-1β has been shown to play a role in local inflammatory and immune mediated diseases, which are associated with local pain. IL-1β, when given systemically or locally, produces hyperalgesia in rats and rabbits. This hyperalgesia is generally thought to be mediated through increased PGE2, but a prostaglandin-independent mechanism is also pointed out. On the other hand, brain astrocytes and microglia are also known to synthesize IL-1, and such brain-derived IL-1 produces a variety of CNS-mediated responses such as fever. To determine whether brain IL-1β affects nociceptive function, we investigated the effect of brain IL-1β (rhlL-1β) on nociception in male Wistar rats. The paw lick latency on a hot plate (50±0.5°C) was measured before and after LCV injection of rhlL-1β (1pg-1μg/kg). LCV injection of rhlL-1β (1-10μg/kg) dose-dependently reduced the paw lick latency. The hyperalgesic action started around 5min after the injection, reached a maximum at 30min and was still observed at 60min. This effect was antagonized by pretreatment with either an IL-1 receptor antagonist, αMSH or salicylate, but not by β-helical CRF. An electrophysiological study performed in urethane (1.2g/kg) anesthetized rats revealed that responses of neurons in the spinal trigeminal nucleus to nociceptive stimuli were elevated by LCV rhIL-1β (10-100pg/kg). At a similar time courses as that of the paw lick latency responses. This enhanced responses of nociceptive neurons was also attenuated by αMSH. The results suggest that brain IL-1β induces hyperalgesia through an activation of arachidonate metabolism, but not by that of CRF system.

425.2


There are a few reports in the literature that interleukin-1 (IL-1) induces analgesia (Nagasawa et al., Eur.J.Pharmacol. 149:49-58, 1988; Bianchi et al., Brain Res.546:139,1991). The present study sought to characterize the analgesic effects of centrally administered IL-1 in rats. In the hot-plate test (HPT), latency to a rear paw lick or a four-footed jump off a 55°C surface was measured; in the cold water tail-flick test (CWT), latency to tail withdrawal from a 3°C liquid was timed. Core body temperature was also monitored with a rectal thermistor. After baseline readings, human recombinant IL-1β (125-2000 U) was injected i.p. (35μg/kg) and post-treatment doses were recorded at intervals from 15 to 180 min. In the HPT, no dose of IL-1 induced greater than 6.1±4.4% maximum possible analgesia (%MPA) at any time point. Similarly, no significant effect of IL-1 on morphine-induced analgesia. In summary, we failed to find any analgesic effect of IL-1, alone or in combination with morphine, at doses which clearly had a physiological effect; this is in contrast to the reports cited above. (NIDA Grants T32 DA07273 and DA 00376)

The expression of interleukin-1β (IL-1β) mRNA in the hippocampus and other brain regions (cerebral cortex, striatum and thalamus) was examined after transient forebrain ischemia using male Wistar rats. IL-1β mRNA was not detected in these regions of sham-operated rats. IL-1β mRNA was induced after transient forebrain ischemia. The induction of IL-1β mRNA had a few peaks, that is, peaks were observed at 30 and 240 min in the four regions examined and an additional peak was observed at 90 min in the striatum.

In addition, the effect of intracerebroventricular injection of IL-1β on neuronal cell death after transient forebrain ischemia was examined in the hippocampus. Seven days after 10 min of ischemia, the number of neurons in the hippocampal CA1 region of saline-injected rats was decreased to 30-40% but the number of neurons in the hippocampal CA3 region did not change, compared with non-treated rats. Injection of IL-1β (30 ng/rat) during the ischemic insult increased the neuronal cell death.

The numbers of neurons in the CA1 and CA3 regions decreased to 10-20% and 30-40% of non-treated control rats, respectively. These results suggest that IL-1β might be produced in the brain after transient forebrain ischemia and was involved in neuronal cell death.


We have reported that i.v. or i.c.v. administration of interleukin-1β (IL-1β) elicited an increase in renal sympathetic nerve activity (RSNA) accompanied with increases in blood pressure, heart rate and body temperature in conscious rats (Kannan et al., Soc. Neurosci. Abstr. 17: 11981, 1991). This study was designed to examine the effects of i.v. injection of IL-1β on discharge pattern in sympathetic nerves innervating the intercapsular brown adipose tissue (IBAT), and compare them with those of RSNA in conscious rats. The IBAT sympathetic nerve activity was increased by IL-1β with an increase in large group discharges synchronous with the cardiac cycle. In contrast, small amplitude activity unrelated to the cardiac cycle increased in RSNA, while large amplitude group discharges decreased. Plasma noradrenaline concentration increased in conscious rats after i.v. injection of IL-1β. The responses were reversed by dexamethasone. The results suggest that IL-1β activates systemic sympathetic outflow via prostaglandins, and modulates the discharge patterns in regional different manner.


The cytokine interleukin-1 (IL-1) has been shown to stimulate release of hypothalamic and pituitary hormones, notably with resulting increase in corticosterone production. The present studies investigate the possible occurrence of the endogenous IL-1 receptor antagonist (IL-1ra) in hypothalamus and paraventricular nucleus at central nervous system, and further analyze the finding of IL-1 receptor (IL-1r) in the pituitary gland. Antisera against synthetic peptides of the human monocyte IL-1α and IL-1β were used for immunohistochemistry, and polymerase chain reaction (PCR) technique was used to detect IL-1β and IL-1α mRNA. In addition, the presence of IL-1ra was examined in the hypothalamus, paraventricular nucleus, and in the pituitary gland. The results indicate that IL-1r and IL-1ra immunoreactive neurons were seen in the paraventricular (PVN) and supraoptic nuclei and in nerve terminals in the median eminence. PCR analysis confirmed the synthesis of IL-1ra in the hypothalamus and in the pituitary gland. The findings indicate that IL-1r and IL-1ra may be synthesized in PVN neurons projecting to the neural lobe. Experiments are carried out to verify this, to identify which populations of PVN neurons express the receptor and functional regulation. This work was supported by grants from the Swedish MRC and Clas Groschinsky's Minnesfond.

We have developed a modified sandwich ELISA to detect the cytokine IL-1β in mouse and rat tissues that utilize a specific rabbit immunoglobulin (IgG) and a polyclonal antibody to the IL-1β peptide. ELISA sensitivity was 1 pg; the inter- and intra-assay variabilities were 6% CV, and the range was 1-2,000 pg. The ELISA detects both mouse and rat IL-1β, exhibits a lower reactivity to human IL-1β and a human IL-1β extended by three amino acids, and shows no cross-reactivity to human IL-1α, human IL1 and rat IL-1 receptor antagonist. Human tumor necrosis factor α, somatostatin, vasoactive intestinal peptide, and a variety of unrelated peptides. Commercially available ELISA amplification kits did not alter the sensitivity but did increase the resolution at the lower peptide concentrations. IL-1β levels in normal mouse were 7.0 ± 1.0 pg/mg tissue and 0.3 ± 0.7 pg/mg tissue in testis; IL-1β levels were detectable in plasma, hypothalamus and hippocampus. Robust increases in IL-1β tissue levels were observed in mouse spleen and testis (6 and 15 fold respectively) six hours after injection of lipopolysaccharide (LPS) (1 mg/kg, i.p.). Similar, albeit smaller changes, were observed in rat spleen and testis six hours after LPS injection (2 mg/kg, i.p.). In summary, we have developed a sensitive and specific ELISA for the detection of IL-1β. This assay should allow the further investigation of the role of IL-1 in regulating brain-endocrine-immune interactions.


Previous studies utilizing IL-1β recombinant human interleukin-1α (IL-1α), IL-1β and IL-1β receptor antagonist have demonstrated high binding sites for IL-1 in the mouse brain and endocrine tissues with characteristics of Type 1 receptors in T lymphocytes. In a preliminary study, there were dramatic species differences in the level of IL-1β binding with high levels of binding present in mouse and rat tissues (hippocampus, spleen and testis), while IL-1α in binding to rat and guinea pig tissues was not detectable. In the present study, we further characterized IL-1 binding in mouse and rat tissues and selective immune cell lines. Utilizing 100 µM or 600 µM IL-1α, IL-1β, IL-1α and IL-1β receptor antagonist were used to determine if high levels of specific binding were observed in EL-4 6.1 cells (representative of Type 1 receptor) and in mouse tissues; however, no specific IL-1α binding was present in Bajj cells (representative of Type II IL-1 receptor) and no IL-1β binding was observed in rat tissues. On the other hand, utilizing 600 µM IL-1β as a ligand, high specific IL-1α binding was shown in EL-4 6.1 and Bajj cells and moderate binding was evident in mouse tissues, whereas specific IL-1β binding to rat tissues was barely within the range of sensitivity of the assay. These data demonstrate that under optimal conditions for labeling Type I or Type II IL-1 receptors, no specific binding is observed in rat tissues suggesting the presence of novel IL-1 receptor(s) in rat tissues.

CARDIOVASCULAR REGULATION: LOWER BRAINSTEM I


CO in the anesthetized rat is dramatically reduced 5-7 minutes after local application of pmole amounts of ET-1 to the ventral surface of the medulla (VSM). VSM application of vehicle or other pressor agents failed to mimic ET-1's effect on CO, suggesting that the drop in CO was a specific effect of ET-1 and not related to changes in medullary perfusion. Changes in CO might reflect changes in heart rate (HR), afterload, preload or cardiac contractility. We are interested in determining how VSM application of ET-1 affected each of these parameters. HR and total peripheral resistance (a measure of afterload) were little changed following VSM application of ET-1. Marked decreases in peak stroke output and left ventricular dP/dt after VSM application of ET-1 suggest a decrease in cardiac contractility. Left ventricular end-diastolic pressure and central venous pressure showed little change following VSM application of ET-1. These data are consistent with the hypothesis that central application of ET-1 decreases CO by causing a sustained decrease in cardiac contractility.

426.2 CENTRAL CARDIOVASCULAR EFFECTS OF JOINING PEPTIDE. T. Hamakubo, M. Yoshida, T. Katauchi, K. Nakajima, R. Mosquera-Garcia, M. Tanigami. Departments of Biochemistry and Pharmacology, Vanderbilt University, Nashville, TN 37232; Peptide Institute, Osaka, Japan

Joining peptide (JP), one of the major products of proopiomelanocortin, is present in the hypothalamus and the pituitary. The biological relevance of this peptide, however, has not been identified. In the present study we characterized the central hemodynamic effects of JP. Rat JP C-terminal amide form (JP) and bovine JP (BP) were synthesized according to the deduced amino acid sequence. Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were anesthetized with urethane and blood pressure (BP) and heart rate (HR) were recorded intravenously. The animals were placed in a stereotaxic frame and the doral surface of the medullas were exposed through a laminar incision or craniotomy. In a group of animals, a catheter was placed into the carotid and to intracerebral administration of either JP or BP (10-50nmol/kg). In a different group of rats, a microcatheter was positioned in the nucleus of the solitary tract (NTS) for microinjection of JP (10 pmol/30 nl). Intracisternal injections of JP in SHR increased mean BP and HR in a dose dependent manner. Maximal effects were seen at 30nmol (22±2 mmHg and 36±10 bpm, n=6). While similar effects were observed in WKY, JP or BP were less potent to increase BP and HR. Microinjection of JP into the caudal region of NTS increased BP by 15±2 mmHg and HR by 37±5 bpm (n=4). Maximal changes in HR occurred at 5 min and recovered within 20 min. The maximal HR response occurred 6 min after microinjection. In 2 of 9 rats the pressor effect was preceded by hypotension and bradycardia. These results suggest that JP is a neuropeptide with important central cardiovascular effects in hypertensive animals.
425.3 AREA POSTEROM STIMULATION INHIBITS RENAL SYMPATHETIC DISCHARGE IN THE ANESTHETIZED RAT. R.B. Felder*, A.L. Pence, L.F. Hayward, College of Medicine, University of Iowa, Iowa City, IA

Area postrema neurons respond to circulating neurohumoral substances and may play an important role in cardiovascular regulation. We examined the effects of area postrema (AP) stimulation on mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) in 8 urethane anesthetized rats with bilateral carotid sinus nerves sectioned. AP was stimulated at low intensity (40 µA, 0.2 ms, 40 Hz) in the midline along its rostral-caudal axis. Penetrations were separated by 0.2 mm. Stimulation was carried out on the surface of AP and at 0.2 mm increments to a depth of 0.8 mm. Stimulation at the surface (n=9) or at 0.2 mm depth (n=1) decreased sympathetic discharge (164±10 to 136±5, 10.2 imp/sec; p<0.05) without affecting blood pressure or heart rate. At 0.2 mm depth (n=8) stimulation elicited significant increases in blood pressure (100±6 to 90±6 mmHg) and renal nerve activity (155±11 to 162±13 imp/sec). There was no change in heart rate. Similar effects were observed when left lateral to midline. Ventral to 0.2 mm, a pressor response was usually elicited and became more pronounced at deeper stimulation sites. Prominent depressor responses were also observed at deeper sites. Thus, the predominant effect of selectively stimulating AP neurons is a modest reduction in MAP and RSNA. These findings support a sympathetic inhibitory influence of area postrema on cardiovascular reflex control.

426.4 AFFERENT NEURONS TO THE ROSTRAL VENTROLATERAL MEDULLA (RVL) IN THE RAT: RELATIONSHIP WITH CALBINDIN-28K IMMUNOREACTIVITY. P.A. Trapp*, H.T. Chang*, Departments of Pharmacology*, Anatomy, and Neurobiology*, The University of Tennessee, Memphis, College of Medicine, Memphis, TN 38135

RVL is an important center for cardiovascular control. Although many RVL neurons co-express calbindin-D-28k (CaBP), a Vitamin D-dependent calcium-binding protein, the relationships between CaBP and the neurotransmitters that project to RVL, and that between CaBP and the RVL afferent neurons are still unknown. In this study, FluoroGold (FG) was injected into RVL to retrogradely label neurons that project to the RVL, and CaBP+ neurons were revealed by Texas Red immunofluorescence. Many FG labeled neurons were found in the brain, including regions that contain many CaBP+ neurons: the nucleus of the tractus solitarius, the medial vestibular nucleus, the contralateral RVL, the central gray, the lateral parabrachial nucleus, the paraventricular nucleus of the hypothalamus, the posterior and the lateral hypothalamus, the substantia innominata, and the central amygdala. Only a minority of FG labeled neurons in these regions were CaBP+. On the other hand, the FG labeled neurons in many of these regions were found in close association with terminal fields immunoreactive for phenylethanolamine N-methyl transferase (PNMT), the sympathetic enzyme. Our results suggest that while CaBP is not a major marker protein in neurons that project to the RVL, CaBP+ neurons are a component of the afferent field of adrenergic fibers, and may be a marker for neurons that are targets of RVL afferent neurons.

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425.5 ROLE OF THE CAUDAL VENTROLATERAL MEDULLA IN REFLEX PRESSOR RESPONSES. J.C. Solomon, A.M. Metcalfe, J.R. Hagell*, M.P. Kaufman, University of California, Div. of Cardiovascular Med., Davis, CA 95616

Previous studies have shown that the ventrolateral medulla plays a role in the central pathway of the reflex increase in arterial pressure evoked by static muscular contraction, peripheral nerve stimulation, and hypoxia. The role of the caudal ventrolateral medulla (CVLM) in these reflex arcs is not clearly defined. We therefore examined the role of the CVLM in these reflex arcs controlling arterial pressure in cholinesterase anesthetized cats. Increases in MAP were evoked by three stimuli: static muscular contraction elicited by ventral root stimulation (CONT), electrical stimulation of the sciatic nerve (SN), and hypoxia (HYF). These changes were compared before and after bilateral microinjections of a broad spectrum glutamate antagonist, kynurenic acid (KYN) (100 mM, 50 nl), into the CVLM. We tested the hypothesis that bilateral microinjection of KYN into the CVLM potentiates the reflex pressor responses to the three stimuli. The mean increase in MAP produced by these stimuli were potentiated within 20-30 min following bilateral microinjections of KYN into the CVLM at sites 1.5 mm dorsal to 0.8 mm rostral to obex. The reflex increase to control levels was either unchanged (n=8) or remained augmented (n=10). In two cats, microinjections of KYN into sites very caudal to obex (-2 mm) elicited either no effect or an attenuation of the reflex increase in MAP. These findings suggest that the reflex arc increasing MAP evoked by static muscular contraction, sciatic nerve stimulation, and hypoxia can be potentiated by blockade of glutamatergic receptors located in the CVLM.

Supported by HL37071.

425.7 SUPRAMELLULAR INPUTS TO CARDIOVASCULAR NEURONS OF ROSTRAL VENTROLATERAL MEDULLA IN MICE. S.K. Agrawal* and E.R. Calvert, Center for Biomedical Research, University of Toronto, Ontario, Canada N5A 5C1 and *Playfair Neurosci. Unit, Toronto Hospital, Toronto, Ontario, Canada MST 2S8

As anatomical and stimulation studies suggest that neurons in the medullary ventrolateral nucleus (RVLM) receive and integrate multiple inputs from supramedullary regions of the brain, experiments were done to test the hypothesis that selective activation of cell bodies in the lateral parabrachial nucleus (LPBN), locus coeruleus (LC) and lateral hypothalamic area (LHA) could excite or inhibit the discharge of neurons in the RVLM. We therefore recorded extracellular activity from RVLM units in urethane anesthetized cats and monitored the changes in firing frequency of these neurons during chemical stimulation of one of LPBN, LC and LHA. Thirtytwo neurons were classified as cardiovascular neurons because their activity was inhibited by baroreceptor activation (1-3 µg phenylphrine i.v.) and displayed a cardiac cycle related rhythmicity. Chemical stimulation with glutamate of arterial pressure sites in the LPBN increased the firing rate (40.3±1.3 %) of 11 (100 %) cardiovascular neurons. Activation of cell bodies in arterial depressor sites in the LC inhibited the firing frequency (59±1.7 %) of 9 (90 %) cardiovascular neurons. Activation of cell bodies in arterial depressor sites in the LHA inhibited the discharge rate (25±4.7 %) of 6 (60 %) cardiovascular neurons, excited one unit and did not alter the discharge rate of the remaining 3 units. There is evidence for convergence of excitatory and inhibitory inputs from neurons located in the LPBN, LC and LHA to cardiovascular neurons in the RVLM.

426.6 VAGAL AND SPINAL MODULATION OF VENTROLATERAL MEDULLA NEURONAL RESPONSES TO REDET STIMULI. M.A. Vazquez*, A. Standish and W.S. Ammons, Department of Physiology, Thomas Jefferson University, Philadelphia, PA 19107

Recent experiments from this laboratory demonstrated that cells within the ventrolateral medulla of the cat respond to electrical stimulation of renal nerves and to activation of renal mechanoreceptors and chemoreceptors. The purpose of this study was to determine if these responses are modulated by vagal or spinal pathways. A total of 1469 cells were studied and neuronal responses were elicited by stimulation of renal nerves from 3.6±0.6 spikes to 2.4±0.4 spikes/s (p<0.05). Vagotomy decreased spontaneous activity of 814 cells from 1.3±0.5 impulses/s (p<0.05), increased spontaneous activity of two cells and did not affect four cells. Bilateral cervical vagotomy increased the evoked response of 5 cells to electrical stimulation of renal nerves from 2.4±1.0 impulses/s to 4.8±1.2 impulses/s (p<0.05). Spontaneous activity of each cell also increased from 1.4±0.9 impulses/s to 3.9±0.9 impulses/s (p<0.05). Bilateral vagotomy never abolished cell responses to renal nerve stimulation. Five cells (26 %) were responsive to renal nerve excitation; 1 cell was also responsive to ureteral occlusion. Vagotomy did not alter the responses. The response to renal nerve stimulation for 9 of 9 cells was completely abolished after spinal cord transection. Following spinal cord transection, the spontaneous activity of 7 cells was not significantly affected. Somatic field classification of each cell was unaltered and cell responses to somatic stimulation above the transection level were comparable or greater. The results of these experiments have demonstrated that renal afferent information to the ventrolateral medulla is conveyed via the spinal cord. In addition, the results are consistent with a tonic modulatory effect of the vagus nerve on ventrolateral medulla cell responses to renal stimulus.

426.8 PARRALLEL AND INDEPENDENT PROGRESSING OF SYMPATHETIC BARS- AND CHEMOREFLEXES IN THE MEDULLA OBONGA. N. Koshiva, D. Haugaa and P.G. Goyant, Dept. of Pharmacology, University of Virginia, Charlottesville, VA 29080

Splanchnic sympathetic nerve discharge (SND), phasic nerve activity (PND) and putative vasomotor sympathetic precursor neurons of the rostral ventrolateral medulla (RVL PMN) were recorded in urethane- anesthetized vagotomized rats without aortic baroreceptor afferents. Cardiac chemoreceptor stimulation (CCS) with brief N2 inhalation increased SND by 101±7, MAP and increased the discharge rate of RVL premonitor neurons by 46±12 % (N=32, 50 range 90% inhibition to 200% activation). During chemoreceptor activation, SND and most RVL neurons displayed pronounced, post-inhibitory rhythmicity. These responses confirm that convergence between peripheral chemoreceptor and baroreceptor inputs to the central network responsible for SND generation occurs at or prior to the RVL premonitor neuronal stage. Bilateral microinjection of Kyn (5 mmol in 100 nl) into RVL blocked the sympathetic chemoreflex but left the sympathetic baroreflex intact (N=6). Conversely, bilateral microinjection of Kyn into the caudal ventrolateral medulla at obex level (RVL) blocked the baroreflex but left the sympathetic chemoreflex intact (N=5). These injections also left intact the ontogenic specificity of the sympathoexcitatory produced by CCS.

The effect of Kyn injection into RVL was blocked to the baroreceptor input by proprioamidula GABAergic cells of RVL with projections to RVL. These cells are likely to be the last mediatory relay of the sympathetic baroreflex before the RVL neuronal stage. The present results suggest that the sympathetic chemoreflex and baroreflex utilize two separate and largely independent channels in the medulla until the RVL premonitor neuronal stage where algogenic synaptic summation of the two inputs probably occurs.

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426.1 DOUBLE LABELING OF RAT SPINAL NEURONS PROJECTING TO BOTH SOLITARY TRACT AND DORSAL COLUMN NUCLEI. C.W. Lim and Z. Wen. Dept. of Neurobiology, Capital Institute of Medicine, Beijing 1000054, China.

Physiological evidence has been presented for spinal dorsal horn neurons with labeled axons terminating in both the solitary tract and dorsal column nuclei (Lim G.W. et al.:Science in China,34:171-183,1991). The present study is aimed at double labeling the cells of origin of the double projection SBN with fluorescent substances.

Propidium (PI) was injected into left solitary tract nuclei (STN) of P3 rat and labeled with biotinylated avidin (Bb). The nuclei were dissected and sectioned. The neuronal cell bodies were identified by biotinylated avidin (Bb). Double labeling with PI and Bb was performed by the avidin-biotin complex method and revealed that these two sets of neurons are different in terms of their functional diversity.


Within a rostrocaudally limited region in the middle of the cuneate nucleus (CN), distinctive biocles of intense CO-activity were observed. The CO-staining in these biocles was approximately 0.3-0.7 mm caudal to the obex, which is located within the previously defined middle region (approximately 0.2-0.9 mm caudal to the obex) that receives a disproportionate large share of primary afferent terminations (Maslany et al., Neurosci. Lett., in press). No CO-biocles were observed anywhere else in the dorsal column nuclei. Transgangliaric labeling (WGA-HRP) demonstrated that several of the CO-biocles of the CN are precisely related to the terminal projection fields of primary afferents from the skin of the forepaw. In contrast, control observations demonstrated that 1) the coarchitecture (Nissl-stained sections), 2) the cytoarchitecture/distribution of microtubule-associated protein 2 (MAP2) and 3) the organization of retrogradely labeled cuneal motorcells did NOT correspond to the topographical organization of the CO-biocles. Postnatal (up to 11 days postpartum) dorsal column transection or removal disrupted the CO-staining pattern in the adult CN.

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The distribution of the calcium-binding proteins, calbindin D28k (CaBP) and parvalbumin (PV), were examined in the dorsal column nuclei and spinohind brain by an avidin-biotin peroxidase method. In the cuneate nucleus (CN), both CaBP-immunoreactive (CaBP-IR) and PV-immunoreactive (PV-IR) cells were found throughout the entire rostrocaudal axis of the nucleus. However, the density of the PV-IR cells was greatest in the previously defined middle region (approximately 0.2 - 0.9 mm caudal to the obex) that receives a disproportionately large share of primary afferent terminations (in press) and is similar to the known distribution of thalamic projection cells. The distribution of the CaBP-IR cells appeared uniform throughout the CN. In the gracile nucleus, where the PV-IR cells were also more numerous, the density of both the PV- and CaBP-IR cells was greatest just caudal to the obex. In the spinal cord, PV-IR cells were found in Rexed’s laminae IV-VI, but in the gracile, both PV- and CaBP-IR cells were found in lamina I-II. A few PV-IR and CaBP-IR cells were observed in the ventral horn. While many PV-IR fibers were found in the gracile tract (GT) in the lumbar region, few PV-IR fibers were detected in the GT in the cervical region, suggesting that a large proportion of PV-IR fibers may be proprioceptive in origin.

427.5 DIFFERENTIAL BRAINSTEM TERMINATIONS OF EXTRINSIC VERSUS INTRINSIC MUSCLE SPINDLE AFFERENTS IN THE MONKEY. C. L. Martin-Ekline* and C. J. Vierck. Department of Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610.

The intrinsic hand muscles, including the lumbricals and the interosseous muscles, appear to play a critical role in fine, coordinated movements of the fingers while the extrinsic hand muscles, located in the forearm may be more important in gross, powerful movements. Differential effects of dorsal column lesions on these two types of hand movements prompted a comparison of the pattern of central terminations from muscle spindle afferents of intrinsic versus extrinsic muscles of the hand. We have injected cholinergic toxin conjugated to horseradish peroxidase (CT-HRP) into a variety of intrinsic hand muscles of the digit I, II, III, and IV to label the spinal cord motor nuclei and motor neurons in the forelimb. Preliminary results suggest that the terminal patterns of extrinsic muscle spindle afferents are distinct from those of intrinsic muscle afferents which are not restricted to the triangularis portion of the main cuneate nucleus and the external cuneate nucleus in the brainstem as muscle spindle afferents of extrinsic muscle terminals appear to be. Supported by NS-17474 and NS-07261.

427.7 OVERLAYING OF NERVE-RELATED AND SOMATOTOPIC ORGANIZATION IN THE CUNEATE NUCLEUS OF PRIMATES. J.K. Baxley and D.J. Wall*. Department of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Sensory fibers from individual fingers, palmar pads, and the somatotopic divisions of the hand terminate in rod-like clusters in the cuneate nucleus. The hand innervation territories of the median, ulnar, and radial nerves do not always coincide with the borders of the somatotopic divisions. How are sensory fiber terminations of these nerves organized in the cuneate nucleus? In the cuneate nucleus of squirrel monkeys following whisker afferent injections of horseradish peroxidase conjugates. The termination field of each nerve is elongated rostrocaudally, and spans multiple somatosensory clusters in the mediolateral and dorsolateral dimensions. Label density varies within the field from "core" regions containing dense, continuous label to "fringe" regions that contain lower density label. These nerve-related termination patterns appear to be consistent with subcortical substrates of nerve dominance columns in the cortical hand map, and provide images of the presynaptic neuropil which loses normal sensory signals after injury of a hand nerve. Supported by NS21105.


In six adult rats WGA-HRP was injected in dorsal root ganglia to label primary afferent terminals (PATs), or in the cuneal spinal cord (6 days after unilateral cervical rhizotomy and lesion of the dorsal quadrant at T1) to label post-synaptic dorsal cuneal terminal in the cuneate nucleus. The anterograde tracer was revealed by TMH histochemistry. Post-embedding immunogold staining was then performed on these sections, using antisera against GABA and for glutamate. PATs are large (mean area: 3.14 ± 0.7 μm2, n = 177), contain many mitochondria and small round vesicles. They are GABAergic at their glutamatergic synapses. In the plane of the section they contact two or more dendrites of various caliber, and receive synapses from small boutons, some GABAergic. The glutamatergic synapse product after injection of the tracer in the spinal cord are small (mean area=1.34 ± 0.7 μm2, n = 185) and contact a single thin dendrite or cell body. They may be GABAergic, or glutamate or negative for either antisera. GABAergic terminals are the smallest of the three types (mean area= 0.9 ± 0.7 μm2, n = 288), and, besides contacting dendrites and cell bodies, they, along with GABAergic, may form covicentric arrangements with other terminals. (NIMH NS 27827 and NIMH 404)
427. 9
ULTRASTRUCTURAL INVESTIGATION ON THE DORSAL THALAMIC OF GUINEA FOG
R. Serapicò*, C. Frascoc, M. De Curtis and S. DeBissi*
* Istituto di Fisiologia e Biochimica Generali, Milano, Italy.
Recent anatomical investigations provided evidence that GABAergic neurons are present in some thalamic nuclei of the guinea pig (Asanuma, 1991). Aim of the present study was to investigate the intrinsic synaptic organization of selected thalamic nuclei such as the ventrobasal complex (VB) and the ventroter nal nucleus (VL), characterized respectively by the presence or absence of corticofugal projections. Adult guinea pigs were perfused under anesthesia with 2.5% glutaraldehyde and 0.5% paraformaldehyde. Vibrato me sections from the thalamus were either processed for immunohistochemical detection of GABA or osmicated and wafer-embedded in Epoxy plastic for ultrastructural investigation. LM confirms that GABA+ neurones are present in VB but not in VL. At EM, several complex synaptic arrangements, similar to those found in the cat, are observed in VB, whereas VL contains only simple axo-dendritic and axo-axonic synapses, as in the rat. The differences in the intrinsic synaptic organization between the two examined nuclei are also consistent with post-synaptic GABA immunolabelling. (II III II 27827)

427. 11
MEDIAL LEMNISCAL TERMINALS IN M. FASCICULARIS: 3-DIMENSIONAL COMPUTER-ASSISTED RECONSTRUCTIONS OF SYNAPTIC RELATIONSHIPS. Harvey J. Rafiunion, Henry L. Raphaelis, Department of Anatomy and the W.M. Keck Foundation Center for Integrative Neuroscience, University of California, San Francisco, California, 94143.
Medial lemniscal axons in the somatosensory thalami of M. fascicularis have been labeled by the anterograde axonal transport of wheat germ agglutinin- horseradish peroxidase (WGA-HRP) following injection of the tracer into the dorsal column nuclei of unanesthetized animals. The tissue was prepared for post-embedding GABA immunocytochemistry and studied by electron microscopy. Computer-assisted reconstruction analysis of serial thin sections was utilized to determine the nature of the synaptic relationship of the terminal and spatial distribution and spatial organization of the projections to the dendritic arbor of thalamocortical relay cells (TCR) and GABAergic local circuit neurons (LCN) and the 3-dimensional features of the synaptic relationship in the terminal. The projections to the axodendritic contact (axon from the proximal dendritic arbor of TCR cells, usually terminating on the first 100μm of these dendrites. In addition, the ML terminals synapse upon the GABAergic dendritic shafts and appendages of LCNs. Counts of contacts of ML afferents upon thalamocortical terminals is more than 90% of the ML terminals contact GABAergic profiles in addition to TCR dendrites. In contrast, the synaptic terminals of STT afferents (STT - adjacent poster) form simple axo-dendritic synapses upon TCR dendrites but are rarely seen to contact GABAergic profiles. 3D reconstruction analysis of ML-M terminal relationship reveals that they form elaborate synaptic arrays with multiple GABAergic dendrites and dendritic appendages of LCNs, the latter in turn, synapsing upon the same element of the TCR dendrite as does the ML terminal. A given TCR dendritic segment receives ML (and no STT) afferent terminals. We conclude that the ML synaptic input upon TCR cells also elicits substantive GABAergic feed-forward modulation of the ML synaptic effect. This supports the hypothesis that the transfer of non-noxious information by primate thalamic nuclei is markedly influenced by GABAergic LCN processing, in contrast to the lack of GABAergic modulation of nociceptive information. Supported by NS-23347.

427. 13
IMMUNOCYTOCHEMICAL COMPONENTS IN THE LEMNISCAL AND SPINOthalamic PATHWAYS IN MONKEY BRAINSTORM: Giuseppe De Biasi, L. Dagnino, L. DeRosa, M. Zanier; Dept. of Morphology, University of Trieste, Italy. 
De Biasi, L. Dagnino, L. DeRosa, M. Zanier; Dept. of Morphology, University of Trieste, Italy.
Cyscnergic nucleus (CO) staining reveals a histochemical parcellation of the principal trigeminal (PV) and dorsal column nuclei (DCN) in monkeys (Norrega and Warr, Brain Res.,363:1389,1982). CO-rich and CO-weak compartments, also defined by immunostaining for calcium binding proteins parvalbumin (PV) and 28kd calbindin (CB) respectively, are also present in the thalamus ventral posterior nucleus (Rausell et al., 1991 J Neurosci.,11:2110,1991). Leptomineral and spinolaminar fibers terminate preferentially in these compartments. Compartmentalization based on CO- and immunocytochemical staining is also a common feature of relay nuclei at other levels of lemniscal and spinolaminar systems.
In M. fascicularis and M. moneu CO-rich compartments in PV are made up of clusters of neurones that show PV immunoreactivity and stain with SMI-14, an antibody to nonphosphorylated neurofilament protein. PV and SMI-32-positive neurones also label the nucleus. CB immunoreactivity occurs in the lateral periphery of PV and in other CO-rich areas that alternate with the CO-rich zones. CB immunoreactive neurones are more numerous in the dorsal part of PV, and no CB axons are observed. DCN show similar CO- PV and SMI-32 staining patterns but few CB neurones are present. PV and SMI-32 staining are present in the medullary lemniscus. In the spinal trigeminal nucleus, CO staining in weaker overall and absent in the dorsal-most portion. PV and SMI-32 staining are confined to the CO-positive and CB staining to the CO-negative regions. 

427. 12
The present work investigates at electron microscopic level the preservation of different areas of the adult isolated guinea pig brain maintained in vitro by arterial perfusion (de Curtis et al. 1991: Hippocam. Res. 1, 341). After characterization of the electrophysiological viability of the preparation via extracellular field potentials, the brain was frozen at different times after isolation (one to seven hours) with a mixed alkaline solution (4% paraformaldehyde and 0.1% glutaraldehyde in PBS, pH 7.2) perfused through the cannnal inserted in the vertebral artery. Samples of neocortex, thalamus and hippocampus were trimmed out from 100 um cortical sections, were canonical and embedded. Semithin and ultrathin sections were then cut, observed at LM and EM respectively and compared to control samples obtained from deeply anesthetized animals directly perfused with the same fixative through the aorta. In all regions explored the tissue was well preserved during the early hours after dissection and showed only minimal and localized alterations after longer incubation times. Cells shapes (somatic and dendritic), myelin sheaths, cytoplasmatic membranes and synaptic clefts in the thalamus and superficial layers of the neocortex showed no major alterations after several hours. Different degrees of extracellular vacuolization (probably due to swelling) and cell damage (membrane shrinkage, cytoplasm vacuolization, darkly stained dendrites, etc.) were observed in the hippocampus and in the deep neocortical layers after five hours of perfusion. These anatomical evidences add new data to the demonstration of the long-term preservation of the isolated in vitro brain.

427. 14
MODELLING SPINDLE-LIKE OSCILLATION IN THALAMOCORTICAL NEURONES. T.J. Tóth* and V. Crunelli, Dept. of Physiology, Univ. Wales Coll. Cardiff, Cardiff CF1 ISS, U.K.
Recent experimental studies have shown that thalamocortical neurones are capable of displaying a variety of oscillatory states that do not require synaptic currents (Leresche et al., 1991). The pacemaker oscillation (i.e., low-frequency oscillation associated with high-frequency bursts of action potentials) has successfully been accounted for by models (McCormick et al., 1992; Tóth and Crunelli, 1992) based on the properties of a low threshold Ca**+ current, I, and inward Na**+K** current, I, that were identified in voltage clamp experiments. Using the same set of synaptic parameters, however, it has not been possible to reproduce the spindle-like oscillation (SLO), a type of activity where low-frequency oscillation occurs intermittently every 5-25 sec. Our model has been extended by taking into account that an increase in intracellular [Ca**+] is known to shift the activation curve of I, towards more positive potentials. This has been able to obtain an oscillatory behaviour that closely resembles SLO and to mimic some of the qualitative properties of SLO observed in vitro. We conclude therefore that I, I, and the leakage current, I, are sufficient to describe SLO. In the intracellular [Ca**+] must be taken into account when dealing with this oscillatory state. 

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427.15

The GABA containing cells in the thalamus consist of local circuit neurons and those of the nucleus reticularis thalami (NRT), which project to most thalamic nuclei. In the rat ventro-basal complex (VB), local circuit neurons make up <0.5% of the total neuronal number. When recording extracellularly from VB thalamocortical cells in a slice preparation containing rat VB and NRT, single shock, low-frequency stimulation of the NRT resulted in an EPSP (due to activation of cortico-fugal fibres passing through the NRT) and a hyperpolarization. The co-application of 20µM CNQX and 100µM DL-AP5 blocked the EPSP to reveal a fast IPSP, which had a time to peak of 10-30ms, reversed at -70mV, and was blocked by bicuculline (10-20µM). A slow IPSP (time to peak of 200-300ms), sensitive to the GABA A antagonist CGP 35348 (100-300µM), was observed. In vitro, for this antagonist, at membrane potentials in the -55 to -65mV range, the decay of the GABA IPSP was able to evoke a low-threshold Ca++ potential, indicating that GABA A receptors may underlie sleep spindle activity in the thalamus. We conclude that the input from the NRT to the VB is mediated via both GABA A and GABA B receptors.

PAIN MODULATION: SPINAL I

428.1

Serotonin (5-HT) is known to be a neurotransmitter in descending pathways. The purpose of the study was to determine the effects of 5-HT on feline dorsal horn neurons. Three cats were anaesthetized with sodium pentobarbital (35 mg/kg). Extracellular potentials were recorded from 7 dorsal horn neurons in the thoracic spinal cord (T1-T3). The axons of 3 of the cells were antidromically activated from the medullary reticular formation. Seven barrelled glass micropipettes were used to pressure eject (100 µm, 50 psi) small volumes (65.5 µL) of 3 concentrations (10 µM, 100 µM, 1mM) of 5-HT (pH 7.3), vehicle (phosphate buffered saline, pH 7.4), and 1 mM d,l-homocysteic acid (DLH; pH 7.3). Microinjections of 5-HT increased the spontaneous activity of all 7 cells and the change occurred within 1 sec. The mean changes in activity for 10 µM, 100 µM and 1mM 5-HT were -0.8, 2.7 and 10.5 spikes/sec, respectively. The excitatory responses to 1mM 5-HT were significantly different from the responses to 10 µM and 100 µM 5-HT. There was no difference in the change in spontaneous activity between the 2 lower concentrations of 5-HT. Five cells were tested for responses to 1mM DLH; 4 were excited and 1 had no effect. No responses to vehicle were found. Using the pressure ejection technique, 5-HT has been found to excite dorsal horn neurons, some of which are spinoreticular tract neurons. (Supported by NIH grant HL29618 and OCAST grant HR-089).

428.2
MODULATION OF SEROTONIN (5-HT) RELEASE FROM SPINAL CORD TISSUE BY OPIOIDS AND KETAMINE. B.K. Kradel, E.H. Stultens, Jr., D.L. Smith, P.J. Monroe, D.J. Smith. Dept. of Anesthesiology, WVU Health Sciences Center, Morgantown WV 26506

Antinociception from some intrathecally administered drugs appears to be mediated in part by spinal serotonergic processes (Pain 12: 57,86; Eur.J.Pharmac. 166: 211:89 & 194: 167:41; Nature 28: 1047:89). In this study, several opioids and ketamine (each in a concentration of 1 µM) were evaluated for their ability to alter the release of 5-HT from superfused spinal cord tissue. Using synaptosomes, it was found that neither fentanyl nor (D-Pen2,5)

In this preparation, and provide further evidence that these drugs do not release 5-HT directly from spinal serotonergic terminals. On the other hand, when a spinal cord slice preparation was used (neuronal connectivity is less compromised), 5-HT efflux was modified by some of these agents. Ketamine, DPDPE and Tyr-Gly-Met-Phen-Gly (DAMGO) enhanced the K+-evoked release of total 5H to 153 ± 13, 190 ± 18 and 178 ± 26% of control, respectively. In contrast, EIC and U50,488 reduced K+-evoked release to 63 ± 14 and 63 ± 13%, respectively. No changes were resolved with either morphine (70 ± 22) or 8-endorphin (105 ± 14%). Thus, these drugs appear to affect neuronal processes that converge on spinal serotonergic terminals. Interestingly, the effect of the various agents on 5-HT release was not always consistent with what would be proposed from the antinociceptive studies that have implicated 5-HT in local spinal actions.

428.3
RELEASE OF ADENOSINE FROM THE SPINAL CORD BY 5-HYDROXYTRYPTAMINE (5-HT) AGONISTS. CHARACTERIZATION OF RECEPTOR SUBTYPES. J. Mason*, D. Leeson, A. Reid, G. Doak and T. White, Dept. Pharmacology, Dalhouse University, Halifax, NS B3H 4H7.

Release of adenosine by 5-HT may mediate a component of the spinal antinociceptive action of 5-HT (Brain Res. 426: 348). In this study, the 5-HT receptor subtypes involved in adenosine release were characterized using behavioral and neurochemical approaches. 8-Phenylthethylphenylacetic acid is a 5-HT agonist but not 5-HT antagonist. Similarly, 5-HT agonists did not release adenosine from dorsal cord spinal cord synaptosomes. In all cases, the adenosine originated from released nucleotide rather than as adenosine per se, as release was reduced by inhibition of 5'-nucleotidase activity. Release of adenosine by 5-HT and CGS 20668 was Ca++-dependent, while that induced by TFMPP and DOX was not. Release of adenosine from the spinal cord appears to be due to activation of a 5-HT receptor subtype; the Ca++-dependent component of release appears to contribute to behavioral effects of 5-HT agonists. (Supported by MRC Canada).

428.4
DESPRAMINE REDUCES NMDA-INDUCED NOCICEPTIVE BEHAVIOUR MEDIATED THROUGH THE SPINAL SHT1A RECEPTOR. N. Meldrum, A. Lund and K. Hole, Department of Physiology, University of Bergen, N-5009 Bergen, Norway.

The mechanism of the antinociceptive effect of despramine (DMI) is unclear. It is generally accepted that excitatory amino acids (EAA's) act as neurotransmitters in primary nociceptive fibers, and in vivo studies have shown that cyclic AMP analogs may influence the NMDA receptor complex.

The modulatory effect of DMI on the nociceptive behavior induced by intrathecal (i.t.) NMDA (0.4 mmol, 7µl) was studied in mice. DMI was administered either i.t. (0.75, 1.5µl) 5 min prior to administration of NMDA. Intraperitoneally (i.p.) (10 mg/kg) 30 min prior to NMDA, or in drinking water during 3 weeks. The nociceptive behavior (biting and scratching) produced by i.t. NMDA was significantly reduced when the animals were pretreated with DMI, either acutely or chronically.

The SHT1A antagonist 8-OH-DPAT inhibits NMDA-induced behavior. A functional upregulation of the SHT1A receptor was found after chronic administration of DMI. We therefore investigated if blocking of the SHT1A receptor would reduce the effects of DMI. A selective SHT1A antagonist, NAN-190 Hydrobromide (10 µg), was injected i.t. 5 min prior to NMDA in animals pretreated with DMI. The SHT1A antagonist reversed the reduction of NMDA-induced behavior produced by DMI.

These findings indicate that both acute and chronic administration of DMI reduce the NMDA-induced nociceptive behavior, and that this may be mediated at least partly through the SHT1A receptor in the spinal cord.
428.5 DIFFERENTIAL EFFECTS OF 5-HT AND NE UPTAKE BLOCKADE ON OPIOD RECEPTOR SUBTYPE SELECTIVE ANALGESIA

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Department of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

Selective antagonism of 5-HT(1A) receptors by pirenzepine, ME-300 or spiroxatrine, or k-opioid receptors produces analgesia through neuroanatomically and neurophysiologically distinct mechanisms. Blockade of 5-HT or NE uptake potentiates morphine analgesia. To determine whether selective 5-HT and NE uptake blockade will potentiate analgesia produced through each of the opioid receptor subtypes, we assessed the analgesic effects of morphine, i.e., DAMGO (mu), i.e., DADLE (delta), U50,488H (kappa), and nalorphine (kappa) in mice treated with zimelidine (10 mg/kg), desipramine (5 mg/kg) or saline using the tail-flick assay. Zimelidine produced a leftward shift in the dose-response curves for morphine, i.e., DAMGO (5-fold), and U50,488H (2-fold). Desipramine produced a leftward shift in the dose-response curves for morphine and i.e., DPDPE (2-fold). These results indicate that a 5-HT may modulate mu, and kappa, analgesia, whereas NE may modulate spinal delta analgesia.

428.8 INTRATHORACIC (I.T.) METOXAMINE POTENTIATES DESMETHYLDOPAMINE SPINAL ANTINOCICEPTION IN THE RAT: EVIDENCE OF A ROLE FOR 5-HT, ADRENERGIC AND ENDOGENOUS OPIOIDS

C. L. M. G. Bolotin, M. D. Madl, E. D. Tarnopol, M. J. Dauvillier, K. D. Proven, K.D. Proven, C. W. van Baal, and P. D. Miller, School of Pharmacy, University of Toronto, Toronto, ON, Canada.

I.t. α-agonists induce selective antinociception (AP) in animals via spinal α-2-agonists, the role of spinal α-2-agonists is less clear. The objectives of this study were to determine: 1) if i.t. methoxamine (MX; α-agonist) potentiates AP induced by i.t. desmethyldopamine (DM), an endogenous antinociceptor in the rat; and 2) if endogenous spinal Met-Enk is involved in this interaction. Male Sprague-Dawley rats (250-450 g) were implanted with i.t. catheters. Rates whose baseline responses and nociceptive function were normal were used in the tail-flick and paw pressure withdrawal tests. All drugs were given i.t. in the following procedure: 10 μg threshold (<10 s) doses of MX to DM yielded an 8-fold leftward shift of the DX dose-response curve (DRC). MX potentiation of DX was not due to a prolonged duration of DX action; and was significantly antagonized by each of the following i.t. pretreatments: prazosin (15 μg), yohimbine (2.5 μg), naloxone (30 μg), or anti-Met Enk (but not by yohimbine preincubated with Met-Enk). Pretreatment with i.t. SCH 32615 (Met-Enk inhibitor) shifted the MX+DX (32615) DRC to the left 6-fold compared to vehicle pretreated rats but had little effect on the DX DRC (no effect on MX). Light microscopy of spinal cord cerebra from rats given MX+DX (twice daily for 3 days) revealed no inflammation, haemorrhage or necrosis, and no changes in substance P- or CGRP-like IR compared to vehicle controls. The data indicate that the MX potentiates DX spinal AP without neurotoxicity. The effect appears to be mediated, at least in part, by spinal Met-Enk, consistent with the known AP interaction between opioid- and α-agonists. (Supported by NRC Canada)

428.9 ALPHA-2-ADRENERGIC AGONIST MEDICATION POTENTIATES ANALGESIC RESPONSES TO OPIOID AND NONOPIOID AGONISTS


The expression of c-Jun, Jun, Jun B, fos B and krox-24 proteins were used to study the antinociceptive effect of ip administered medetomidine (MED), a selective α2-adrenergic agonist, in the rat CNS. Protein levels were detected by immunocytochemistry. MED (100 or 300 μg/kg) was injected 12 min before the injection of formalin (F: 5% 50 μl) in the plantar skin of the hindfoot. The rats were killed and perfused 90 min later, Atipamezole (ATI; 1.5 mg/kg ip) was used to reverse the effects of MED. For induced expression of all studied proteins in the spinal dorsal horn ipsilaterally, and in the medial thalamus. Both MED doses strongly (80-90%) suppressed the expression of all proteins, but not in thalamic neurons. ATI completely reversed the effect of MED. Thus, the expression of immediate-early gene (IEG) encoded proteins is under powerful control of α2-α2-adrenoceptors in spinal but not in thalamic neurons. These IEG data dissociate from recent behavioral and electrophysiological findings (Perrotta, et al. Neurosci.1991.44.705) indicating that in the thalamus also the low but the spinal high MED dose produced antinociception. (Supported by DFG)

428.10 REINFORCEMENT OF SPINAL MORPHOAMNEnergic NEUROTRANSMISSION INDUCES ANTIINOCICEPTION IN THE RAT TAIL FLCK TEST - ONE ADDITIONAL MODE OF ACTION OF THE CENTRAL ANTAGONIST "TRAMADOL".

M. S. Reina, R. H. Schulz, and F. Schneider.

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Tramadol has low affinity for opioid receptors and shows norepinephrine (NE) and serotonin uptake inhibition in standard assays. We tested whether NE blockade occurred at spinal sites and whether it can effect antinociception. Slices of the rat dorsal spinal cord were preincubated with NE, then superfused and stimulated electrically twice. Tramadol enhanced the stimulation-evoked overexpression of trivia, starting at 1 μM, qualitatively similar to the NE uptake blocker desipramine (DMI). The effects were mainly due to the (-)-enantiomer of tramadol. When uptake sites were blocked by DMI, DMI's time-effect was lost, suggesting NE uptake inhibition is responsible for tramadols effects. I.t. injection site of 12 μg tramadol at the lumbar produced a significant effect in the tail flick test; both enantiomers were roughly equally potent. Antinociception was also observed with i.t. DMI, starting with 6 μg. Effects of DMI and tramadol were antagonized by i.p. injection of 1 mg/kg yohimbine. The results from this and in vitro and in vivo experiments provide evidence that tramadol reinforces NE neurotransmission at the spinal site and that this mode of action in addition to opioid-like activity is involved in spinal antinociception.
428.12 MUSCARINIC CHOLINERGIC SPINAL ANTIINOCICEPTION. E.T. Iwamoto*, L. Marion, N.W. Pedigo, and R.D. Guarino, Department of Pharmacology, University of Kentucky College of Medicine, Lexington, KY 40536

Intrathecal (i.t.) administration of the muscarinic cholinergic agonist, (+)-cis-methylycoline (CCL), into the lumbar spinal cord of male Sprague-Dawley rats produced antiinociception as assessed by the P2° C hot-plate and tail-flick (7 sec control latency) assays. i.t. CD induced antiinociception within 5 min for up to 3 hr without altering other motor reflexes or median effective i.t. dose of CD was ~10 nmol in both antiinociceptive assays. Tissue content of CD 30 min after an i.t. injection of 37.5 nmol H-CD was estimated at 1 μM. The antiinociception induced by 37.5 nmol of CD was antagonized by 5 min i.t. pretreatment with pirenzepine or methotrimeprazine at IC50 doses of approximately 1 and 7 nmol, respectively. Five min i.t. pretreatment with 20 nmol LY-53857, 25 nmol 5-(-)zacopride, or 25 nmol idazoxan (doses which were inactive alone) each partially antagonized 37.5 nmol CD antiinociception, whereas buffer, mecamylamine or naloxone had no effect. CD inhibited H-QNB binding in spinal cord homogenates with μmolar affinity. The data suggest that i.t. CD antiinociception may be mediated via M1 and/or M2 muscarinic, 5-HT2 and/or 5-HT3 receptor sites. (Supported in part by NS 28847 and KTRB.)


This study investigated the effects of aging on the antiinociceptive efficacy of the μ opioid agonist DAMPGO. Young, mature and aged Fischer 344 rats (6, 16 and 26 months old, respectively) were injected intrathecally (i.t.) with various doses of DAMPGO. Opiate-induced changes in tail-flick latency (TFL) and nociceptive withdrawal latency (NWL) were recorded 5, 15, 30, 60 and 120 min post-injection. The results demonstrated that DAMPGO dose-dependently elevated TFL and NWL in each of the three age cohorts. When the tail-flick test was used as the nociceptive measure, the potency ratio of DAMPGO was significantly different between the 16 and 26 month old rats. No significant age-related differences were observed in the potency ratio for DAMPGO when the hot plate was used as the nociceptive test. Thus, while higher i.t. doses of DAMPGO are apparently needed to produce antiinociception in older rats, the μ opioid receptor site continues to mediate spinal DAMPGO-induced antiinociception throughout the life span of the rat.

428.15 SELECTIVE INHIBITION OF NON-PEPTIDE SUBSTANCE P ANTAGONIST CP-96,345 ON NOCICEPTION IN RATS. Y.C. Gosed, S.P. Rabby, J.S. Mitchell, R.S. Cohen and J. Sagen, Departments of Anatomy and Cell Biology and Anesthesiology, University of Illinois College of Medicine, Chicago, IL 60612.

Substance P (SP) may mediate nociceptive transmission via the NK1 receptor in the spinal cord. Studies in this field have been limited by the lack of specific antagonists. A recently developed non-peptide SP antagonist, CP-96,345, is specific for the NK1 receptor. The purpose of this study was to assess the effects of this antagonist on nociception. Nociception was determined using tail flick, paw pinch and hot plate tests in rats. The rats received intrathecal injections of various doses of CP-96,345 and pain sensitivity was assessed at several times before and following injection. In addition, the ability of CP-96,345 to inhibit SP-induced biting and scratching was assessed. Results from analgesiometric testing indicated that doses up to 240 μg had no significant effect on either tail flick latency or paw pinch threshold. Hot plate latency was only elevated by high dose of the antagonist. In addition, the SP antagonist failed to inhibit the SP induced biting and scratching. The Intrathecal injection of CP-96,345 produced a dose-related decrease in tail skin temperature, significant only at the NK1 receptor. Preliminary binding studies using [3H]-SP were also performed. Assessment of the ability of CP-96,345 to inhibit the binding of [3H]-SP to the rat spinal cord and rat salivary tissue indicated that CP-96,345 and SP behave similarly at the NK1 receptor. The results of this study suggest that, while CP-96,345 binds the SP site at the NK1 receptor in the spinal cord, this receptor is most likely not involved in mediating some types of acute pain at the spinal cord level. (Supported in part by NS 25054.)

428.14 EFFECTS OF INTRATHECALLY INJECTED NON-PEPTIDE SUBSTANCE P ANTAGONIST CP-96,345 ON NOCICEPTION IN RATS. Y.L. Gosek, S.P. Rabby, R.M. Mitchell, R.S. Cohen and J. Sagen, Dept. of Anatomy and Cell Biology and Anesthesiology, University of Illinois College of Medicine, Chicago, IL 60612.

Substance P (SP) may mediate nociceptive transmission via the NK1 receptor in the spinal cord. Studies in this field have been limited by the lack of specific antagonists. A recently developed non-peptide SP antagonist, CP-96,345, is specific for the NK1 receptor. The purpose of this study was to assess the effects of this antagonist on nociception. Nociception was determined using tail flick, paw pinch and hot plate tests in rats. The rats received intrathecal injections of various doses of CP-96,345 and pain sensitivity was assessed at several times before and following injection. In addition, the ability of CP-96,345 to inhibit SP-induced biting and scratching was assessed. Results from analgesiometric testing indicated that doses up to 240 μg had no significant effect on either tail flick latency or paw pinch threshold. Hot plate latency was only elevated by high dose of the antagonist. In addition, the SP antagonist failed to inhibit the SP induced biting and scratching. The Intrathecal injection of CP-96,345 produced a dose-related decrease in tail skin temperature, significant only at the NK1 receptor. Preliminary binding studies using [3H]-SP were also performed. Assessment of the ability of CP-96,345 to inhibit the binding of [3H]-SP to the rat spinal cord and rat salivary tissue indicated that CP-96,345 and SP behave similarly at the NK1 receptor. The results of this study suggest that, while CP-96,345 binds the SP site at the NK1 receptor in the spinal cord, this receptor is most likely not involved in mediating some types of acute pain at the spinal cord level. (Supported in part by NS 25054.)

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242. 17

EVALUATION OF THE EFFECT OF NITRIC OXIDE ON THE RELEASE OF ICRP FROM THE DORSAL HORN OF THE SPINAL CORD IN RATS.
M.G. Gary*, H.E. Geier, and K.M. Hargens. Univ. of Minnesota, Dept. of Restorative Sciences, and Dept. of Pharmacology, Minneapolis, MN.

Nitric oxide (NO) is a gaseous, biologically active molecule that plays a role in numerous physiological processes. In this study, we evaluated the effect of NO on the release of ICRP (i.e., substance P) from the dorsal horn of the spinal cord in rats.

NO was administered to rats via intrathecal injection, and the release of ICRP was measured using an in vitro radioassay. The results showed a significant increase in the release of ICRP following NO administration, indicating that NO plays a role in the release of ICRP from the dorsal horn of the spinal cord in rats.

242. 18

REGULATION OF TACHYKININ RECEPTOR mRNA EXPRESSION IN RAT DORSAL HORN DURING NOCICEPTION. K. E. McCarron and J. E. Krause. Depnt of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The tachykinin peptide family includes substance P (SP) and neurokinin B (NKB). These peptides are both contained in the dorsal horn of the spinal cord and are involved in the modulation of nociception. In this study, we investigated the regulation of tachykinin receptor mRNA expression in the dorsal horn during nociception.

The dorsal horn was isolated from rats, and the expression of tachykinin receptor mRNAs was measured using reverse transcription-PCR. The results showed that the expression of tachykinin receptor mRNAs increased during nociception, indicating a role for these receptors in the modulation of nociceptive responses.

242. 49

PAIN MODULATION: SPINAL I

Monday PM

429. 1


Activation of nociceptors, peripheral inflammation, or noxious pressure elicits excitability of spinal cord neurons. We investigated the activity of dorsal horn cells during hyperalgesia induced by UV radiation in rats.

The rats were divided into two groups: a control group and a UV-induced hyperalgesia group. The UV-induced hyperalgesia group was exposed to UV radiation, while the control group was not. The activity of dorsal horn cells was recorded using a microelectrode technique.

The results showed a significant increase in the excitability of dorsal horn cells following UV radiation, indicating that UV-induced hyperalgesia increases the excitability of spinal cord neurons.

242. 9

PAIN MODULATION: SPINAL II

Wednesday PM

429. 2

USE OF FOS-LIKE IMMUNOREACTIVITY TO STUDY THE EFFECTS OF ANALGESIC COMPOUNDS IN SPINAL CORD NEURONS OF POLYARTHRITIC RATS. C. Abadie and J. M. Boussert. INSERM U161 and EPHE, 2 rue d’Alkma, 75014 Paris, France.

We have recently shown that during the development of adjuvant-induced arthritis (AIA) in the rat, the number of SP-immunoreactive (SP-IR) neurons in the lumbar spinal cord was maximal 3 weeks after induction of the disease, which is the acute phase of hyperalgesia. The number of SP-IR neurons increased by a factor of 4 in the AIA group compared to the control group.

In this study, we investigated the effects of analgesic compounds on the number of SP-IR neurons in the lumbar spinal cord of polyarticular rats. The rats were divided into four groups: a control group and three experimental groups treated with different analgesic compounds. The results showed that the number of SP-IR neurons was significantly reduced in the experimental groups compared to the control group, indicating a role for analgesic compounds in the modulation of nociception.

242. 49

THE ROLE OF ACTIVATION OF SPINAL NEURONS IN TONIC PAIN AND EDEMA DEVELOPMENT AFTER FORMALIN-INDUCED TISSUE INJURY. A. C. Durrant and T. Wheeler. Anesth. Dept. of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140, USA.

Behavioral and electrophysiological studies of the response to formalin (FL) in rats suggest that neuronal changes associated with the early phase may be required for full expression of the tonic nociceptive responses. Here, saline (2%) or formalin (0.25%, 1%, 5%) was injected into 70-90 g male rats at either -10 min or 10 min after FL (0.25%, 5%) into the right hind paw (n=8).

Formalin-induced hyperalgesia (FL, 0.25%) was recorded in the right hind paw at 1 min, dose dependent function and increases 50% no-water foot lick latency only transiently (~15 min), but when it is given in 2% formalin is significant and prolonged spontaneous hyperalgesia. Moreover, 0.25% formalin produces a biphasic response in rat paw, whereas 2% formalin induces only slight hyperalgesia.

In contrast, post-FL latencies have a more significant effect than FL exposure, which results in more foot lick latencies, after which formalin gradually recovers to control levels. Although FL, it intrinsically reduces development of edema (3-5 h). However, formalin exposures cause significant reductions in hyperalgesia. Our data suggest that, immediately after the barrage of primary afferent firing impinging on spinal neuronal sparsus supraspinal reorganization of spinal response is initiated. These changes may also be associated with the development of inflammation as well as pain-related behaviors. Since brief protection of spinal neurons significantly reduces the tonic response, our findings suggest the use of local anaesthetics to prevent the development of functional neuronal changes in response to tissue injury (DA90365 & DA97237).
429.5 DORSAL HORN NEURONS SERVING THE LOW BACK ACTIVATION BY SYMPATHETIC STIMULATION. R.G. Gillette, R.C. Kramis, and W.J. Roberts*. Dept. of Neurosurg, & R.S. Dow Neurologic. Sciences Inst., Good Samaritan Hospital & Medical Center, Portland, OR.

Most somatosensory afferents in the extreme lateral dorsal horn of L4-S in cats have receptive fields in the lumbar region and/or hip and proximal hindlimb. They are hyperconvergent WDRs which receive input from all or most of the following tissues: paraspinal muscles, fast joint capsules, vertebral periost, spinal dura, disc, hip and proximal leg muscles, and skin. Such neurons are likely to subserve low back pain.

Clinical reports indicate that sympathetic blocks reduce low back pain in some patients, suggesting a segmental sympathetic role. We therefore tested somatosensory neurons in the lateral lumbar spinal cord for responding to electrical stimulation of the lumbar sympathetic trunk. Single unit recordings were made from L4-S in adult pentobarbital anesthetized cats. Of 82 neurons with conus and/or deep receptive fields in the lumbar region, 70% were responsive to sympathetic stimulation (SS). Excitatory responses to SS were differentiate into: a) short-latency, enhanced responses; and b) longer-latency, non-enhanced responses. The former were resistant to systemic alpha-adrenergic antagonists and are thought to result from activation of afferent fibers in the sympathetic trunk, some of which innervate paraspinal tissues. The non-enhanced responses were attenuated by alpha antagonists and are thought to result from direct or indirect sympathetic activation of afferents. The results indicate that both somatosensory afferent and sympathetic efferent activity in the lumbar sympathetic trunk can contribute to low back pain.

429.8 NEUROKININ RECEPTOR ANTAGONISTS MODIFY THE RESPONSES OF PRIMATE STT NEURONS TO CUTANEOUS STIMULI. W.D. Willis*, J. Palecek, V. Paleckova, J. Ragland, and W.D. Willis. Department of Neuroscience and Anatomy, 200 University Blvd., University of Texas Medical Branch, Galveston, Texas 77550-2702.

We explored the effects of two second messenger systems, the inositol phosphatase cascade (activated by trans-ACPD) and the protein kinase C pathway (activated by phorbol ester, PMA) on the responses of STT cells to cutaneous and chemical stimuli. The experiments were conducted in 5 young adult monkeys (Macaca fascicularis). STT neurons were identified by antidromic activation and recorded using a carbon dioxide electrode in the center of a multibarrel array, the outer barrel of which contained extracellular amino acids (EAAs). The responses of the STT cells to cutaneous stimulation (BRUSH, PRESS and PINCH) and to noxious chemical stimuli (HCl) and acrolein were studied. The responses of the STT cells to cutaneous stimuli were enhanced, i.e., CAP and PMA produced a decrease in response latency. In contrast, the responses to noxious chemical stimuli were suppressed. The enhancement of cutaneous responses by trans-ACPD was suppressed by TTX, indicating that the enhancement was due to the activation of the transmembrane calcium channel. The enhancement of noxious chemical responses by trans-ACPD was enhanced by TTX, indicating that the enhancement was due to the activation of the transmembrane calcium channel. The enhancement of noxious chemical responses by trans-ACPD was suppressed by TTX, indicating that the enhancement was due to the activation of the transmembrane calcium channel. The enhancement of noxious chemical responses by trans-ACPD was suppressed by TTX, indicating that the enhancement was due to the activation of the transmembrane calcium channel.

429.9 EFFECTS OF SYSTEMIC MORPHINE ON LAMINA I NEURONS IN THE CAT. L.P. Serrano* and A.D. Craig. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

The action of morphine on lamina I spinothalamic tract (SCT) cells, which form half of the STT, has not been determined. Previous work demonstrated that morphine enhances the activity of "cold"-sensitive lamina I STT cells. This study further tests the hypothesis that morphine's actions are organized in the belief by examining its effects on nociceptive lamina I units. Units recorded with tungsten microelectrodes in L7 in barbiturate-anesthetized cats were characterized with natural stimuli and with antidromic activation from the thalamus. L7 lamina I neurons that identified with PHA-L. Quantitative heat and pressure stimuli were used to measure the effects of increasing doses of systemic morphine (0.1-2 mg/kg iv) on the responses of 10 nociceptive lamina I cells (6 STT, 3 non-STT, 1 unclassified) that had receptive fields in the ventral hindpaw. All cells had graded sensitivity to noxious heat. Morphine inhibited the responses of 9 cells (mean=54% of control), including all 6 STT cells. Seven of 10 STT lamina I cells was inhibited and 25 non-STT cells were enhanced by low doses (154%). Morphine in higher doses suppressed the responses of 2 non-STT WDR neurons to heat but not to pinch. Naloxone reversed the effects in 7/8 cells. These observations are consistent with the claim that lamina I STT cells form an integral component of the central representation of pain, since all such cells were inhibited by morphine. However, these results also suggest that some STT lamina I neurons are not morphine sensitive, which supports the hypothesis that opioidergic modulation of lamina I neurons is facioally organized. (Supported by DA 07402)

Discipline in mice (MK-801), a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, has been shown to reduce thermal hyperalgesia in rats with hindpaw inflammation. Other excitatory amino acid (EAA) receptor antagonists were examined to further elucidate the involvement of NMDA receptors in the hyperalgesia associated with inflammation. Hyperalgesia, assessed by paw withdrawal latency (PWL) to a heat stimulus, was induced by a single injection of carrageenan (CARRA) (6.0 mg) into the hindpaw of rats. The effects of NMDA receptors were examined by intrathecal (I.T.) injection of the EAA receptor antagonists: (2)-aminoo-5-phosphono-pentoic acid (AP-5), (2)-3-(2-carboxyphosphatase)-propyl-1-phosphonic acid (CPP), MK-801, ketamine hydrochloride (KET), 7-chlorokynuric acid (7-CK), and 6-cyano-7-nitroquinolxaline-2,3-dione (CNQX). Whereas PWLs of non-injected paws and those of naive rats were not significantly affected, PWLs of injected paws were dose-dependently elevated. The rank order of potency of these agents to reduce hyperalgesia was: MK-801 > AP-5 > CPP > 7-CK = KETA >> CNQX > 0. In contrast, I.T. injection of the opioid receptor agonist, [D-Ala2,MePhe4,Gly-ol5]-enkephalin (DAMGO, μ-selective) produced antinociception in both injected and non-injected paws. DAMGO was about 66 times more potent than MK-801. The dose-dependent effects of various NMDA receptor agonists provide additional evidence that NMDA receptors are involved in CARRA-induced thermal hyperalgesia.

429.12 DIFFERENTIAL ROLES OF NMDA AND NON-NMDA RECEPTOR ACTIVATION IN INDUCTION AND MAINTENANCE OF THERMAL HYPERALGESIA IN RATS WITH PAINFUL PERIPHERAL MONONEUROPATHY. D.J. Mayer*, A. Macl, J.L. Hazl, J. Lat*, and D. Price*. 60892.

Dept. of Anesthesiology, Medical College of Virginia, Richmond, Virginia 23298. "Division of Neurosurgery, University of Texas, Houston, Texas 77025. Exciatory amino acid (EAA) receptor activation within the spinal cord has been implicated in neuropathic pain following nerve injury. Using a rat model of peripheral mononeuropathy (Bennett and Xie, Pain, 1988, 33:87), we compared the effects of intrathecal treatment with NMDA receptor antagonists (MK801) and H/A/66) and a non-NMDA receptor antagonist (CNQX) on induction and maintenance of thermal hyperalgesia induced by chronic constrictive injury (CCI) of the ligated sciatic nerve. Four daily saline treatments with 20 nmol H/A/66 or CNQX beginning 15 min prior to nerve ligation (pre-injury treatment) reliably reduced thermal hyperalgesia in CCI rats on days 3, 5, 7, and 10 after nerve ligation. Thermal hyperalgesia also was reduced in CCI rats receiving a single gastroinjury with H/A/66 (20 or 80 nmol) or MK801 (5 or 20 nmol) on Day 3 after nerve ligation when thermal hyperalgesia was well developed. In contrast, a single post-injury CNQX (20 or 80 nmol) treatment failed to reduce thermal hyperalgesia or to potentiate effects of H/A/66 or MK801 on thermal hyperalgesia. Moreover, multiple post-injury CNQX treatments utilizing the same dose regimen as employed for the pre-injury treatment attenuated thermal hyperalgesia only when the treatment began 1 or 24 hrs (but not 72 hrs) after nerve ligation. The results suggest that NMDA and non-NMDA receptor activation may have differential roles in induction and maintenance of thermal hyperalgesia following constriction nerve injury and that mechanisms underlying post-injury neuropathic pain may be associated with EAA-mediated central alterations. Supported by NIH grants NS 24009.


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NMDA (1 nmol-1 pmol) was administered through an 8.5 cm intrathecal (I.T.) catheter to the lumbar spinal cord of awake Sprague-Dawley rats. Changes in tail-flick (TF) latency in response to noxious heat were monitored 0.5, 1, 2, 5, and 10 min post-drug. Lower doses of NMDA (100 fmol-1 pmol) produced a dose-dependent facilitation of the TF reflex (maximal at 0.5-1 min) while greater doses (0.25 nmol-1 pmol) produced a dose-dependent decrease in the TF reflex (maximal at 2-5 min), and also produced a caudally-directed scratching and biting behavior and vocalizations (CBSV). Maximal facilitation was produced by 10 pmol NMDA while maximal inhibition and CBSV were produced by 1 nmol NMDA. Pre-treatment with D-APV (1 fmol-1 pmol, I.T.), which produced no change in baseline TF latency, blocked all NMDA-produced effects in a dose-dependent manner (100 fmol D-APV produced maximal blockade for about 40 min). D-serine (10 nmol-1 pmol, I.T.) and glycine (10 pmol-1 pmol, I.T.) produced a dose-dependent facilitation of the TF reflex which was comparable to the facilitation produced by NMDA (10 pmol); the facilitatory effects of NMDA and D-serine were blocked by 7-chlorokynurenic acid (10 pmol-10 pmol, I.T.). The NMDA-produced biphasic effects on TF latency, but not the CBSV were similar in lightly pentobarbital-anesthetized rats and produced a similar facilitation at 10 mg/kg. In non-anaesthetized rats completely abolished the inhibition of the TF reflex produced by 1 nmol NMDA while the facilitation produced by 10 pmol NMDA remained unchanged.

In summary, these data suggest that while NMDA-produced facilitation of the TF reflex may be a local phenomenon, inhibition of the TF reflex and CBSV may be produced by activation of descending pathways that in turn engage a descending inhibitory pathway to produce inhibition of the TF reflex.

429.14 HYPERESTHESIA INDUCED BY SPINAL GLUTAMATE AND NK-1 TACHYKININ RECEPTORS IS REDUCED BY INTRATECHAL NON-NTRITERAL ANTI-INFLAMMATORY DRUGS (NSAIDs). A.B. Malhotra and T.L. Yaksh*. Dept. of Anesthesiology, UCSD. La Jolla, CA 92035-0818.

Spinal administration of NSAIDs can significantly diminish the second phase, behavior induced by subcutaneous injection of formalin in the paw. Based on the growing appreciation of the involvement of excitatory amino acids (EAA) and substance P directly into the spinal cord. Intrathecal injection of NMDA (7 nmol), AMPA (1 nmol) and substance P (7 nmol) each resulted in a dose dependent decrease in the latency of the response evoked by a thermal stimulus applied to the hind paw. The selective antagonists MK-801 (29 nmol), CNQX (77 nmol) and PSN,1,345 (330 nmol), respectively, given 10 min before the agonists, blocked the observed thermal hyperesthesia for the respective agonists. Importantly, injection of the antagonists 10 min after the agonists failed to block the decreased paw withdrawal. To assess the potential role of prostaglandins released by the spinal activation of the respective receptors, we examined the effects of several NSAIDs on the evoked thermal hyperesthesia. We found that acetazolamide (100 nmol), ketorolac (75 nmol) and indomethacin (25 nmol) resulted in a complete reversal of the induced thermal hyperesthesia. Importantly, the intrathecal NSAIDs were equally effective in reducing the thermal hyperesthesia whether administered before or after the agonists. Additionally, the inactive stereoisomer R-73480 failed to show any effect at doses 10 times higher than the active isomer S-73480. This emphasizes that the NSAIDs action were mediated by an effect upon cyclooxygenase. These data provide evidence that activation of spinal glutamate and NK-1 receptors results, at least in part, in local generation and release of prostaglandins, which mediate a spinal facilitation of the animal's response to noxious stimuli. (Supported by NIH DA 02110, T.LY)


Prostaglandins(PGs) influence the development of hyperalgesia in the periphery. However, centrally their role is not yet well defined. This experiment was designed to investigate the influences of intrathecally administered PGE2 and PGF2α on behavioral responses to non-noxious, and noxious somatic and visceral stimuli in rats. Intrathecal catheter was implanted at the level of L2-L3 in rat. The tail flick (TF) test and colorectal distension (CD) test were employed to measure the responses to noxious somatic and visceral stimuli, respectively. Alpha adrenergic (A2) to mechanical pressure produced by three kinds of Semmes-Weinstein monofilaments (SWMs) ranging 0.08 g to 2.33 g was further significantly increased following administration of both PGF2α and PGE2 and the effects lasted for at least 2 days, while in the saline group there was no significant difference. This result suggests that PGs may act on hyperalgesic processing in the spinal cord.
430.2 RESPONSES IN THE RAT TO NOXIOUS COLORECTAL DISTENSION IN THE PRESENCE OF ACETIC ACID. M. Burton* and G.P. Gehart
Department of Pharmacology, University of Iowa, Iowa City, IA 52242.

The mechanisms of visceral pain have recently been a major focus of investigation. Distension of hollow organs has been shown to be noxious to the body. Clinically, there is usually some pathology associated with pain from visceral organs; however, this has not been extensively studied in animals. Therefore, the aim of this study was to determine the effects of acetic acid irritation on the responses to noxious colorectal distension (CRD).

Pre- and post motor (EMG) responses to CRD (80 mmHg, 20 sec, 3 min apart) were examined in unanesthetized, chronically instrumented, male Sprague-Dawley rats before and 6 and 24 hours after 1 ml intracolonic 5% acetic acid (or vehicle). The results of a separate group of rats were examined 4, 6, 8, or 24 hours after 1 ml intracolonic acetic acid for evidence of leukocyte infiltration. Acetic acid did not produce any change in resting mean arterial pressure (MAP), or in the MAP in response to CRD at any time tested. In contrast, the magnitude of both baseline EMG and ΔEMG produced by CRD were significantly increased at 6 and 24 hours after acetic acid, as noted in the table. Acetic acid had no effect on the number of leukocytes in colonic tissue at any time tested. These results suggest that irritation, but not inflammation, of the colon produced by acetic acid results in an increase in the magnitude of nociceptive responses to CRD.

control 6 hr 24 hr
Baseline EMG (%) 100 170 ± 66 255 ± 99
ΔEMG (%) 100 182 ± 47 216 ± 34

430.4 EFFECTS OF MANIPULATING INTRACELLULAR CALCIUM ION CONCENTRATION USING VOLTAGE SENSITIVE CALCIUM CHANNEL BLOCKERS AND DANTROLEN ON PERSISTENT HINDLIMB FLEXION IN SPINALIZED RATS. M.S. Anderson and J.T. Baradelli
Department of Psychiatry, St. Elizabeth's Hospital of Boston, Boston, MA 02135 & Department of Pharmacology, University of New England, Biddeford, ME 04005.

Stimulation (2mA, 7ms, 100Hz, 60min) across the musculature of the upper hindlimb in the spinalized rat produces a persistent hindlimb flexion. Previously, we have shown that NMDA receptor antagonists interfere with the induction of this flexion. Given NMDA receptor stimulation indirectly may activate voltage sensitive calcium L-channels, the dose response of pretreatment with nimodipine, nifedipine and diltiazem on induction of a persistent hindlimb flexion was examined.

Following spinalization of adult Long Evans rats under halothane anesthesia, animals were pretreated with one dose of a particular test agent (nimodipine: 0.03, 0.1, 0.3, and 1.0mg/kg ip, nifedipine: 0.1, 0.3, 1.0, and 3.0mg/kg, ip, diltiazem: 0.03, 0.1, 0.3, and 1.0mg/kg, ip) and wound clips were applied to the musculature of the right upper hindlimb. After a 30min waiting period, current (2mA, 7ms, 100 Hz, 1 hr) was delivered across the clips. In all groups, flexion was reduced in a dose dependent manner. Since 1) the systemic administration of calcium L-channel blockers may promote skeletal muscle relaxation and 2) NMDA receptor activation may effect release of intraneuronal calcium ion stores, the dose response of pretreatment with dantrolene sodium which theoretically inhibits the release of both skeletal muscle and neuronal calcium ion stores was studied. Following the above protocol, dantrolene sodium (0.1, 0.3, 1.0, and 3.0mg/kg, ip) pretreatment reduced poststimulation flexion in a dose dependent manner. These data suggest that induction of a persistent hindlimb flexion in the spinalized rat depends on both adequate intraneural and skeletal muscle intracellular calcium ion levels and 2) activation of skeletal muscle afferents. (Support: Mead Johnson & St. Elizabeth's)

430.6 ENDOGENOUS ANTI-ALGALERGIA: EFFECTS OF ANYGILADA, DORSAL RHAPHE & SPINAL LENSES L.B. Watkins*, E.P. Wier telak, K. Mooney-Herber ger, R. Grun & S.F. Maier,
Dep. Psychology, University of Colorado at Boulder, Boulder, CO 80309.

We have shown that rats can learn to activate neural circuitry that blocks opiate analgesia (Wiertelak et al., Science, 1992). Using classical conditioning procedures, "danger" signals (cues predicting shock) come to elicit conditioned analgesia & "safety" signals (a light cue predicting that shock will NOT occur for some period) come to elicit conditioned anti-analgesia. To begin defining anti analgesia, we tested the anti analgesic effects of central nucleus of the amygdala (CeA; bilateral), dorsal raphe nucleus (DRN) & spinal dorsal funiculus & bilateral DRN & sham controls. Each of these areas has been previously implicated in fear &/or analgesia.

After surgery, rats received training to contextual "danger" signals & the discrete light "safety" signal (see Wiertelak et al., Science, 1992). Tail flick (TF) latencies were recorded during each of the 4 days of conditioning to assess the effect of the various lesions on acquisition of conditions of analgesia & anti-analgesia. Conditioned analgesia was then extinguished across 4 days. All rats were injected with morphine the next day to test whether any lesion would prevent reversal of morphine analgesia.

The results were as follows. CeA lesions (a) blocked development of conditioned analgesia, yet (b) did NOT block shock safety signal reversal of intra-thalamic morphine analgesia. DLP lesions (a) blocked development of conditioned analgesia yet (b) prevented reversal by the safety signal of both conditioned analgesia & 5 mg/kg s.c. morphine analgesia. Taken together, these data provide strong evidence that anti-analgesia is mediated by neural circuitry different from that previously defined for analgesia systems. NIMH 3T2MH14617-15; K.M.H. supported by the Hughes Fund.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
430.7 INTERNAL AVERSIVE STIMULI ELICIT ACUTE & CONDITIONED HYPERALGESIA. E.D. Wierstel*, R. Mooney-Heiberg*, W. Van Wyhe, G.J. Piatellie II, S.P. Makar & L.F. Watkins. Department of Psychology, University of Colorado, Boulder, CO 80302. Hypoalgesia aversive to external stimuli promotes effective defense/escape behaviors. Perhaps, then, the adaptive response to aversive internal stimuli is hypoalgesia, to promote recuperative behaviors. An emotive agent (0.15 M inhum chloride [LiCl]) was used to produce an aversive internal stimulus. Indeed, LiCl produced pronounced hyperalgesia (tail flick), compared to controls. This appeared specific for nociceptivity, as sensitivity to non-palpable stimuli (vony) hairs was unchanged. LiCl-induced hyperalgesia involves activation of a centrifugal pathway since it is blocked by spinal transection. Further, this effect appears to be mediated by cholecystokinin (CCK) since it is abolished by prolylamic (0.4 mg/kg s.c.), a CCK antagonist. Paradoxically, 7 mg/kg naloxone produced profound hypalgesia, but not in vehicle control rats.

Since illness clearly could produce hyperalgesia, could hypealgesia be conditioned to cues associated with illness? To address this question, 2 groups were tested using a taste aversion paradigm. The first received saline paired with LiCl. The second (control) received equal numbers of LiCl & saccharine exposures, but in an unpaired fashion. On test day, all rats received saccharine. The rats that previously had saccharine paired with LiCl showed profound hypalgesia, compared to controls. Conditioned hyperalgesia was again abolished by prolylamic, indicating a role of CCK. Again, paradoxically, naloxone produced profound hypalgesia in the rats that previously had saccharine paired with LiCl.

These results suggest that conditioned effects on pain sensitivity are bidirectional. External danger cues can produce analgesia. The present data demonstrate that aversive internal stimuli can produce analgesia as can learned cues that signal such events. Supported by NIH MH33241H14617-15, K.M.H & W.V.W. supported by the Hughes Fund.

430.9 MILD SHOCK PRODUCES AN UNCONDITIONED, NALTREXONE-INSENSITIVE INCREASE IN REACTIVITY ON THE VOCALIZATION MAGNITUDE AND THRESHOLD TESTS. P.A. C. Parada & J.W. Grau. Dept. of Psychology, Texas A&M Univ. College Station, TX 77843.

This work suggests that an aversive event can produce either an increase (hyperalgesia) or decrease (hypolgesia) in pain reactivity. Although stress-induced hyperalgesia has been extensively explored both at the behavioral and neural levels, relatively little is known about stress-induced hyperalgesia.

Here, we first establish a paradigm that produces a robust increase in reactivity to pain. Subjects were trained to receive either mild shock (0.5 - 1.0 mA), never anything, or nothing. Subjects were trained to receive either mild shock (0.5 - 1.0 mA), never anything, or nothing. The paradigm resulted in a substantial increase in vocalization threshold (hyperalgesia) that decayed over time (Experiment 1) and a decrease in vocalization threshold (hypolgesia) when vocalization was blocked (Experiment 2).


This experiment was performed to compare the effects of capsaicin and its new derivatives on the tail-flick and jaw opening reflex evoked by noxious stimuli and content of Substance P in spinal dorsal horn and trigeminal sensory nucleus. Tail-flick reflex was tested by hot water immersion method in rat after subcutaneous injection of capsaicin, parado, shogao and demethoxy-NE (DM-NE). Neural conduction of peripheral sensory nerve was determined in saphenous nerve. Saphenous nerve was exposed and action potential was evoked by noxious electrical stimulation. Capsaicin and its derivatives were dissolved in vehicle and applied to saphenous nerve between stimulating and recording sites for 30 minutes. Amplitude and conduction velocity of action potential was compared before and after application of drugs. Substance P of spinal cord and trigeminal sensory nucleus was determined by radioimmunosassay. Capsaicin inhibited neural conduction of mainly C-fiber but parado and shogao had inhibitory effect on both Aβ- and C-fiber. Capsaicin and DM-NE significantly increased latent period of tail-flick reflex at first day after injection. Capsaicin decreased the substance P in spinal dorsal horn and central part of trigeminal sensory nucleus.

430.11 A COMPARISON OF NOCICEPTIVE RESPONSES USING TWO TEMPERATURE THRESHOLD VERSUS REFLEX LATENCY. Premyslaw Mark1, Anpolary L. Vavassorop 1, Bogdan Sadowski1 and John C. Liebeskind1. 1Dept. of Psychology, UCLA, CA 90024, 2Dept. of Psychology, U. of Orleans, LA 70148, and 3Institute of Genetics and Animal Breeding, Jastrzebie, Poland.

Mice bred for high (HA) and low (LA) stress-induced analgesia displayed differences in baseline hot-plate latencies. Interpretation of baseline sensitivity in the hot-plate test, however, is confounded by the stressful nature of this test and it is unclear whether such differences are due to stress-induced analgesia (produced by the test itself) or due to differences in tonic pain inhibition. We have recently developed a method of measuring hot-plate pain sensitivity which reduces the stress component. HA and LA mice were habituated for 20 minutes to a hot-plate maintained at 37±1°C. The temperature of the hot-plate was then gradually increased and the temperature values at which pain reflexes occurred were found in baseline temperature thresholds for HA and LA mice. In contrast, HA mice showed higher baseline responses than LA mice when the cold-plate was used (i.e. plate maintained at 56±1°C). Morphine produced a dose-dependent analgesia in both tests. These results suggest that this method of measuring absolute temperature thresholds would be a simple, effective method of assessing pain sensitivity, which greatly reduces the confounding effects of stress. Supported by NIH Grant NS07628 and an Unrestricted Research Grant from the Bristol-Myers Squibb Company.

430.12 EXPOSURE TO A HEAT STRESSOR INDUCES AN OPIOID CONDITIONED HYPOALGESIA IN RATS TESTED FOR NOCICEPTION TO FORMALIN. P. Fosse and R.F. Westbrook. School of Psychology, University of W.S.W. Sydney, Australia.

Rats exposed to a heated floor in a distinctive environment (EI) and subsequently tested there on that floor are hypoalgesic. This conditioned hypalgesia is naloxone irreversible and not cross-tolerated with morphine. However, morphine-tolerant rats come to acquire the conditioned hypalgesia. The display of conditioned hypalgesia with the initial exposure to the heated floor enhances the acquisition of the conditioned hypalgesia. The present study examined whether this conditioned hypalgesia was naloxone irreversible and not cross-tolerated with morphine. The conditioned hypalgesia induced by formalin was also naloxone irreversible and not cross-tolerated with morphine. However, the conditioned hypalgesia induced by tail-flick was naloxone reversible and cross-tolerated with morphine. These results, thus, suggest that the nature of the conditioned hypalgesia is determined by the pre-exposure to a heat stressor which may depend upon the type of pain elicited on test. Although acquisition of the conditioned hypalgesia may depend on exposure to the heated floor with naloxone, morphine-tolerant rats were hypalgesic in the formalin test. However, morphine-tolerant rats were just as hypoalgesic as morphine-naive ones when tested with the opioid in the formalin test. Thus, the capacity of morphine tolerance to block the acquisition of conditioned hypalgesia appears to be independent of opioid pain mechanisms.
430.13 NOCICEPTIVE RESPONSES TO HIGH AND LOW RATES OF FOOT SKIN HEATING IN RATS MAY BE MEDIATED BY DIFFERENT NOCICEPTORS. D.G. Yeomans, V. Fitz, and H.K. Proudfit. Department of Pharmacology, U.I.C., Chicago, IL 60612.

The selective actions of capsaicin or morphine to increase or decrease nociceptive responses mediated by polymodal nociceptors were used to test the hypothesis that heating the dorsal hairy surface of the hindpaw at low rates elicits withdrawal responses evoked by polymodal nociceptor activation, whereas high rates of heating brake these responses mediated by other nociceptors. Capsaicin was applied topically to the hairy foot skin of rats to selectively sensitize polymodal nociceptors. Capsaicin reduced the latency of the reflexive withdrawal response evoked by low heating rates, but not by high heating rates.

In addition, the skin temperature at which the response to low heating rates occurred was reduced from 46 to 41°C, but the temperature at which the responses to high heating rates occurred (91°C) was not affected.

Responses mediated by the activation of C polymodal nociceptors have been demonstrated to be preferentially attenuated by low doses of systemic morphine (Cooper et al., Pain, 24:93-116, 1986). In the present experiments, low doses of morphine (0.01 to 1.0 mg/kg i.p.) selectively attenuated nociceptive responses to low heating rates in normal rats, and to an even greater extent after capsaicin treatment. The same doses of morphine did not attenuate nociceptive responses to high heating rates in either normal rats or those with capsaicin sensitized skin. Higher doses of morphine (2.0 to 10.0 mg/kg i.p.) attenuated responses to both types of stimulation. These results support the conclusion that low rates of heating elicit responses elicited by the activation of polymodal nociceptors. In addition, the potency of analgesic drugs, determined using thermal methods, may depend on the rate of skin heating. Supported by USPHS Grants DA03980 (HFP) and DA05406 (CCY).


Touch-evoked pain, mediated by Aβ fibers, can be produced in the following ways: C-fiber activation by intradermal capsaicin (Torendijk et al., 1992), J Pain., 44:94, 1994. In the rat, peripheral blockade of inhibition in the spinal cord results in touch-evoked aggravation (Yaksh, 1989, Pain, 37, 117). We set out to examine the effects of C-fiber conditioning and central disinhibition on responses in the decerebrate spinal rat. The response of hamstring motorneurons to noxious and non-noxious stimuli applied to the receptive field was tested. In control conditions tactile or stimulation caused little or no activity. Responses to repeated standardized touches of the hindpaw or electrical activation of Aβ-afferents were greatly enhanced after C-fiber were activated either by cutaneous application of mustard oil or by conditioning the sural nerve (1 Hz, 20-s trains of 1118 ± 24.8 and 4272 ± 48.5 s, respectively). A similar facilitation of touch-evoked responses was observed following a subcutaneous dose (3 μg) of strychnine or bicuculline. i.t. (+1042 ± 499 and +1239 ± 377%, respectively). These changes were associated with a decrease in the mechanical threshold of the flexor reflex and an increase in the response to noxious stimuli. Sural conditioning at 1 Hz (10 Hz, 20 s) had no effect. These results suggest that C-fiber induced central sensitization may be involved in allodynia as well as hyperalgesia and that disinhibition may be involved.

431.1 SYNAPTIC ORGANIZATION OF DOPAMINERGIC AMACRINE CELLS IN THE LARVAL TIGER SALAMANDER RETINA. R.A. Glazebrook, R.B. Walter, and C.B. Watford. Alice R. McNprhon Laboratory of Retina Research, Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX, 77380.

Immunocytochemistry of tyrosine hydroxylase (TH) was used to visualize larval tiger salamander dopaminergic amacrine cells. The avidin-biotin immunoperoxidase method was used to immunostain TH immunoreactive cells in vibratome-prepared sections that were routinely processed for ultrastructural examination. TH-positive somas exhibited an evenly distributed peroxidase reaction product throughout their cytoplasm. Their nuclei were generally unstained and possessed indented nucleated membranes. TH-positive processes were generally stained throughout and lacked synaptic vesicles. TH-positive processes were generally stained throughout and lacked synaptic vesicles. TH-positive processes were generally stained throughout and lacked synaptic vesicles. TH-positive processes were generally stained throughout and lacked synaptic vesicles. TH-positive processes were generally stained throughout and lacked synaptic vesicles.

A total of 112 synaptic relationships were observed in the inner plexiform layer that involved TH immunoreactive processes. TH-positive processes were presynaptic to amacrine cell processes (33.0%) and to processes that lacked synaptic vesicles (26.9%). Synapses onto amacrine cell processes were observed only in superlayer 1. Synaptic contacts onto processes that lacked synaptic vesicles were observed primarily in sublayer 1 (93.4%), but also in sublayers 3 (3.3%) and 5 (2.9%). As post synaptic elements, they formed conventional synaptic contacts that were characterized by symmetrical synaptic membrane densities.

431.2 COEXISTING RELATIONSHIPS OF SUBSTANCE P-AMACRINE CELLS IN THE LARVAL TIGER SALAMANDER RETINA. C.A. Fogarty, A. I. Newlin, and R.B. Walter. Alice R. McNprhon Laboratory of Retina Research, Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX, 77380.

In situ hybridization for Substance P (SP) immunocytochemistry was combined with either immunocytochemistry of gamma-aminobutyric acid (GABA) or autoradiography of high-affinity GABA uptake to examine for the presence of GABA in SP-amacrine cells of the larval tiger salamander retina. These thousand SP-like immunoreactive cells were visualized in double-label preparations. Double-label analyses revealed two populations of SP-amacrine cells that express each marker of GABA activity. Both populations were activated either by cutaneous application of mustard oil or by conditioning the sural nerve (1 Hz, 20-s trains of 1118 ± 24.8 and 4272 ± 48.5 s, respectively). A similar facilitation of touch-evoked responses was observed following a subcutaneous dose (3 μg) of strychnine or bicuculline. i.t. (+1042 ± 499 and +1239 ± 377%, respectively). These changes were associated with a decrease in the mechanical threshold of the flexor reflex and an increase in the response to noxious stimuli. Sural conditioning at 1 Hz (10 Hz, 20 s) had no effect. These results suggest that C-fiber induced central sensitization may be involved in allodynia as well as hyperalgesia and that disinhibition may be involved.


Correlation of electrophysiological, morphological, neurochemical characteristics and connectivity of neurones is important for understanding the functional organization of neuronal systems. The aim of this study is to gain some insight into how the above characteristics can be brought together in a model system. Amacrine cells were electrophysiological recorded and then injected with a solution of 4% bicytin in 0.5 M KCl.

Bicytin diffused through gap junctions and often up to 25 coupled amacrine cells were observed from a 2 min injection at 1-10 nA. From the analysis of the stained networks, it appeared that not all coupled amacrine cells were morphologically the same. Some cell groups were cytochrome oxidase and processed for GABA immunohistochemistry. Several cells generating off-transient responses have yielded positive results. However, it was not clear whether all coupled cells within a network were GABAergic. Present research is further analysing the morphological and neurochemical characteristics of amacrine cell networks.


Many types of retinal amacrine cells show homologous coupling when injected with the biotinylated tracers, biocytin or Neurololitin, thus revealing their somatic array (Vaney, 1991). Moreover, these small tracers are readily transported along the thin processes that characterize "axon-rearing" amacrine cells: visualization of these long processes is greatly aided by photochromic intensification of the DAB reaction product in the presence of tetrazolium salts (Vaney, 1992). We have thus used Neurobiotin injections of microscopically identified cells in the superfused rabbit retina to characterize the complete morphology and the somatic mosaic of several types of low density, axon-rearing amacrine cells. They include the long-range cell (LR; Vaney et al., 1988), the type 1 and type 2 catecholamine-accumulating cells (CA1 & CA2; Tauchi et al., 1990, the type 1 NADH-diaphorase cell (ND1); Sagar, 1990), the interstitial amacrine cell (PA1; Famiglietti, 1992a), and the presumptive somatostatin cell (PA2; Famiglietti, 1992b).

Whereas the LR, ND1 and PA1 cells showed strong homologous coupling, the PA2 and CA1 cells were only weakly coupled, and the CA2 cell showed no coupling. The LR and PA1 cells showed homologous coupling to somata located in the inner plexiform layer of the rabbit ganglion cells layers. Both the LR and ND1 cells also showed homologous coupling to other types of presumptive amacrine cells. The distal processes of the LR cells and the boot dimen low NMD to result from the soma: this greatly exceeds the extent of other retinal interneurons that have been intracellularly labelled by previous methods.

Genetic ablation of gamma-2 crystallin expressing cells leads to photoreceptor death. We have previously demonstrated a decreased density of neurons in the Ganglion Cell Layer in transgenic microphotic mice as a result of this embryonic thymus, which prompted the idea of the normal density of DCaM, 2° dendritic field diameter and 3° dendritic morphology. The diameter of DCaM somata in normal CD-1 mice ranged from a low of 3.0 μm to a high of 22.4 μm with an average of 13.1 ± 3.9 μm. The dendritic field diameters range from normal CD-1 mice at a low of 70.7 ± 27.9 μm. Transgenic microphotic DCaM ranged from a low of 10.24 μm to 26.3 μm and had an average of 16.3 ± 3.8 μm. The dendritic field diameters in these animals ranged from a low of 63.7 μm to a high of 234.9 μm and had an average of 136.8 ± 50.0 μm. These data correspond to an increase in approximately 24% and 93% respectively. These data indicate that DCaM dendritic fields are capable of altering their morphology in response to changes in their local environment. Supported by NEI 01426.

431.7 CHARACTERIZATION OF LIGHT-EVOKED NMDA RECEPTOR-MEDIATED INPUT TO GANGLION CELLS IN DARK-ADAPTED RETINAS. I.S. Diamond and D.R. Copenhagener. Bioengineering Graduate Group, Departments of Physiology and Biophysics, University of California, San Francisco, CA 94143.

Light-evoked synaptic input to ganglion cells has been shown to be mediated by both NMDA and AMPA-type glutamate receptors (Mitman et al. 1990). Pfltzer Phys. 175: 184.

We have used standard whole-cell patch clamp techniques in the tiger salamander retinal slice to study the time course and magnitude of the light-evoked glutamate receptor-mediated inputs to ganglion cells in dark-adapted retinas. In voltage-clamp recordings, the reversible effect of D-2-amino-7-phosphonoheptanoic acid (AP7), a competitive NMDA antagonist, on the response to a second flash of light was greater at -40 mV, a potential at which NMDA-mediated currents were near to maximal; the effect was less at -90 mV, where the NMDA receptor channel was blocked by external Mg2+ (1 mM). The relative strength of the NMDA-mediated input with respect to the AMPA input was determined by applying AP7 to the retina at -40 mV to -90 mV. AP7 (30 μM) caused as much as a five-fold reduction in this ratio.

Mitman et al. showed that cells in light-adapted preparations displayed a relatively fast rise time. Rapid synaptic inputs could be discriminated temporally, because the dark-adapted input had a much slower time course (time to peak = 100 msec) than the AMPA input (time to peak = 25 msec). In dark-adapted retinas the ganglion cell response to dim light had a much slower time course (time to peak = 500 msec). When AP7 was applied, the magnitude of the light-evoked current at -40 mV decreased, but a significant change in time course was seen. Neither the magnitude nor the time course of the dim flash responses at -90 mV was affected by AP7. Consequently, there is considerably more temporal overlap of the AMPA- and NMDA-mediated light responses in dark-adapted than in light-adapted retinas. Any nonlinear interactions between the AMPA and NMDA conductances would, therefore, be enhanced under dark-adapted conditions.


Middle (M) and long wavelength sensitive (L) cones, which constitute approximately 90% of the foveal cone population, can be distinguished from short wavelength sensitive (S) cones by differences in immunoreactivity to antigens, eye type, and ultrastructure. However, M and L cones have not so far been distinguished from each other, and it is not clear from their relative numbers nor their circuitry have been determined. We reasoned that if the two synapses that link each cone to its pair of midget ganglion cells (on and off) differ in the numbers and types of connections between each cone and the midget ganglion cells, it might be possible to distinguish between the two classes. In electron micrographs of serial sections, we identified, for a small patch of cones (outer segments at 1.5° nasal), the pair of midget bipolar cells which each cone connected to and which connected to the corresponding midget ganglion cells. For one set of cones (N=17), the number (mean ± s.d.) of synapses outputs (ribbons) per midget bipolar cell was 36 ± 2, and the number of synapses inputs per midget ganglion cell was 26 ± 2. Thus, in the case of the ganglion cells, the ratio of 1.4 was close to the expected ratio of 1.5. For another set of cones (N=9), the number of synapses outputs per midget bipolar cell was 51 ± 5, and the number of inputs per midget ganglion cell was 26 ± 2 in 3. Thus, in the case of the midget ganglion cells, the ratio of 2 was similar to the expected ratio of 1.5. It is possible that the ratio of 1.1 to M cones is about 2, as consecutively the two classes identified here, represent, respectively, L and M cones. Supported by EY08124.

We thank P. Sarnatich, A. Meyers, and L. Chioseki for their help.


We have used a one dimensional version of the reverse correlation procedure of Jones and Palmer (J. Neurophysiol., 55: 1187-1211, 1987) to examine the spatio-temporal (ST) response profiles of cat retinal W-cells and compare these with those of X- and Y-cells. The stimuli consisted of a circular patch of bright or dark bars which are presented for a fixed duration, typically 50 msec, at one of 16 positions spanning the entire receptive field along one spatial dimension. A series of stimuli are presented successively with no interstimulus interval, and both the position and contrast (bright or dark) of each stimulus are varied randomly. The times of occurrence of all action potentials occurring during the presentation sequence are recorded and the reverse correlation procedure quantifies the relationship between the time of occurrence of each spike and the position, contrast and time of onset of each stimulus in the sequence. The resulting ST-profiles thus represent one dimension of space and one of time, and provide an estimate of the cell’s spatio-temporal impulse response. Most tonic W-cells have ST-profiles similar to those of X- or Y-cells, i.e., a spatial center-surround organization at short latencies, which is space-time inseparable due principally to the longer latency of the surround response. Some phasic W-cells also conform to this pattern, but some have profiles that are much simpler, consisting of a single central zone, not offset in space or time by an antagonistic region, while others have much more complicated profiles, often with central zones which are bimodal in the space domain, flanked by antagonistic zones that are offset in both space and time. Supported by NIH EY08038.

431.10 HYPEROSMOTIC ACTIVATION OF TRANSMITTER RELEASE FROM PRESYNAPTIC TERMINALS ONTO RETINAL GANGLION CELLS. W. Wu and R. F. Miller. Department of Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

We studied neurotransmitter from 2nd order neurons to ganglion cells, using micro-hypertonic stimulation (500 mM) to activate transmitter from presynaptic terminals in the inner plexiform layer. These experiments were carried out in a perfused retinal slice preparation of the tiger salamander, using direct visualization under an upright binocular microscope. A micropipette, filled with hypertonic sucrose (0.5 M + Ringer) was placed in the IPL and whole-cell recordings were obtained from single ganglion cells. Single cells were studied under current and voltage-clamp conditions, and many cells were stained with 0.1% 6-carboxyfluorescein added to the whole-cell pipette and visualized with fluorescent microscopy. Brief positive pressure pulses (0.5-2 bar), applied to the sucrose pipette, activated a postsynaptic response in ganglion cells which was graded in magnitude, based on the duration of the pulse pressure. Voltage-clamp analysis and pharmacological studies indicate that the pH increase is sufficient to activate both excitatory and inhibitory components. When the inhibitory components are blocked with picrotoxin (100 μM/strychnine (10 μM)), the post synaptic current can be studied as a relatively pure hypertonic post synaptic event which typically consists of a combination of non-NMDA and non-NMDA contributions based on the use of AP7, an NMDA receptor antagonist and NBIQ (2,3-Dialkylamino-6-nitro-7-sulfonylbenzo (F) quinoline), a non-NMDA receptor antagonist. This approach permits the analysis of relative NMDA/non-NMDA contributions into identified cell types and at different spatial positions along the soma-dendritic tree. Supported by NIH grants ROI NEI 67376 and NS 17763 awarded to RPM.
341.11
STRUCTURE AND FUNCTION OF GANGLION CELLS PROJECTING TO THE CAT'S GENGULATE WING: AN IN VITRO STUDY. M. Pu, T. Parr and D.M. Benson. Div. Biology & Medicine, Brown Univ., Providence, RI, 02912

The cat's gengulate wing (retinorecipient zone of the pulvinar) is thought to receive its retinal input from a single morphological class of presumed W-cells termed spongy cells (Leventhal, et al., JCN 194:90; Rodeck and Watanabe, Neurosci, Absr. 12:90). We labeled wing-projecting cells by retrograde transport of fluorescent beads and studied their morphology and physiology in vitro. Intracellular staining with Lucifer Yellow and biocytin showed that nearly 100% of these spongy cells (17.2-about 24 µm) had the medium-sized somas (17.2-µm dia., mean 24 µm) and large, radiate dendritic fields typical of episomal cells. Dendritic-field diameters increased with eccentricity in the central retina (500 µm near the f.o.c.; 500-1000 µm at 3-mm eccentricity) but exhibited little further increase more peripherally (500-1000 µm). Many dendritic profiles were ellipsoidal with long axes pointing toward the a.c. Dendrites stratified narrowly in the inner IPL. Axons were as thick as any among presumed W-cells and similar to those of beta cells (= 1 µm dia.). The morphology of these episomal cells closely matches that of a wide-field type prominent among W-cells and similar to that of beta cells (= 1 µm dia.).

Supported by EY08108 and a Sloan Foundation Fellowship to DMB.

341.12

At present, it is difficult to correlate the ganglion cell types distinguished by function with those distinguished by morphology and central projections. However, straightforward techniques are available to:
1. determine functional properties by means of extracellular recording from ganglion cells in vivo, and 2. determine morphological and central destinations by means of a retrograde label and intracellular injection (e.g. HRP, Neurobiotin) of ganglion cells in vitro. We have developed an approach that combines these two techniques with in vivo imaging so as to allow the receptive-field properties, dendritic morphology, and central destinations of individual ganglion cells to be characterized.

Near-infrared fluorescent microspheres (Crimson, Molecular Probes) are used as the retrograde label, in order to preserve rod sensitivity. Labelled ganglion cells are visualized by what is, in effect, an epifluorescence ophthalmoscope. This device makes use of a barrier filter, a 135 mm imaging lens, and a CCD array cooled to -45 °C in order to allow the slow collection of the image of the ganglion-cell layer, using a relatively dim, monochromatic excitation beam. Spatial resolution is 2 µm/pixel; intensity resolution is 12 bit (0.025%).

Image quality is good, with contrast between labelled cells and background as large as 3:1. Images may be saved on Tungsten-In-glass recording electrodes with Crimson microspheres in the tip can be advanced onto selected labelled cells, and the apparatus swung out of the way in order to investigate receptive-field properties. Later, the same cells can be located and intracellularly injected via the in vitro technique. Supported in part by NIH grants EY02923 and EY01730, and by the E.K. Bishop Foundation.

341.13
NEURAL CIRCUITRY OF FOVEAL GANGLION CELLS IN THE HUMAN RETINA. Hyla Kolb* and Jill Crooks. Physiology Dept., University of Utah, Salt Lake City, U.S.A.

While the relationship of midget bipolar cells and midget ganglion cells is well established (Kolb and DeKorver, '91) little is known of the synaptic circuitry involving amacrine cells to ganglion cells in the primate fovea. Thus in this study we have concentrated on understanding the neurochemical signature of the input amacrine cells to ganglion cells using techniques of postembedding immunocytochemistry for GABA and glycine on EM serial sections.

Both midget and parasol ganglion cells and their respective input bipolar cells have been included in the analysis. GABAergic amacrine cells provided about 27% of the amacrine input to ON-center ganglion cell dendrites while glycineric amacrine provided only 6% of the synapses. OFF-center cells received about the same proportion of GABAergic synapses (23%). OFF-center cells received a higher proportion of glycineric synapses (23%). 50 to 60% of the amacrine synapses to all types of ganglion cells were of unknown neurotransmitter species.

GABAergic upon bipolar axon terminals formed about 50% of all the amacrine input for both ON-center and OFF-center variety. Only in a minority of cases was the same GABAergic amacrine postsynaptic to the bipolar presynaptic upon the ganglion cell dendrite. In contrast, GABAergic amacrine cells were frequently reciprocal upon the bipolar cell without making a synapse upon the ganglion cell. There is evidently a complex synaptic circuitry concerning bipolar/ganglion/amacrine interactions even to construct the most "simple" foveal ganglion cell receptive fields. (Supported by grant EY03323)

341.14

Recent biochemical and electrophysiological studies have shown that both polyamines and glycine can enhance the activity of the NMDA receptor. This work was undertaken to determine if polyamines and glycine can further enhance the strength of the excitatory synaptic inputs to retinal ganglion cells.

Excitatory synaptic currents were recorded from ganglion cells in the tiger salamander retinal slice preparation using whole-cell patch recording. Synaptic inputs were elicited by puffing KCl onto the dendrites of bipolar cells presynaptic to the ganglion cell. The AMPA component of the input was isolated by including 1.5-MgCNQX, 1.5 μM strychnine and 100 μM picrotoxin in the bath. We investigated whether or not a polyamine site was associated with NMDA receptors on ganglion cells using the polyamines against diaminodicarboxylic acid (DA10), DA10, at 10-50 μM, reversibly reduced the amplitude of the NMDA component of the synaptic input. The competitive polyamines antagonist diethylstilbestrol reverses the suppressive action of DA10, consistent with DA10 acting specifically at the polyamine receptor. These results suggest that polyamines can modulate excitatory synaptic inputs to ganglion cells.

We used the glycine modulatory site antagonist 7-CI-kynurenate to determine if glycine could modulate the NMDA component of synaptic input. 7-CI-kynurenate (20 μM) reversibly reduced the puff-elicited NMDA component of the synaptic input to ganglion cells. These results suggest that a glycine site associated with the NMDA receptor complex on ganglion cells can modulate the strength of their excitatory synaptic inputs.

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341.15
EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE ON GANGLION CELLS IN THE RABBIT RETINA. Ralph J. Jensen. Southern College of Optometry, Memphis, TN 38104.

The putative neurotransmitter vasoactive intestinal peptide (VIP) has been shown to be present in a population of amacrine cells. Like the neurotransmitter dopamine, VIP stimulates adenylyl cyclase activity in the rabbit retina. In the present study, we examined the effects of bath-applied VIP on ganglion cell recordings extracellularly in a superfused, rabbit retinal preparation.

Most OFF-center and ON-center ganglion cells responded with an increase in spontaneous activity when VIP was applied to the retina. The light responses of these cells to spots and annuli were however only minimally affected. When applied to retinas bathed with the dopamine antagonist (+)-SCH 23390, VIP enhanced the light responses of most OFF-center ganglion cells. VIP brought out both the center and surround responses that were reduced by (+)-SCH 23390. The effects of (+)-SCH 23390 on OFF-center ganglion cells were not reversed by VIP. It is proposed that VIP counteracts the effects of (+)-SCH 23390 on OFF-center ganglion cells by stimulating adenylyl cyclase in dopamine-sensitive cells of the retina. (Supported by NIH grant EY07318).

341.16
Prenatal Development of Neuropeptide Y Immunoreactivity in the Ganglion Cell Layer of the Cat Retina Jeffrey J. Hustler* and Leo M. Chalupa, Psychology Department and the Center for Neurobiology, University of California, Davis.

Previously we have shown that immunoreactivity for neuropeptide Y (NPY-IR) identifies a subgroup of gamma type ganglion cells in the adult cat retina which project to the superior colliculus (Hustler et al., 1991). In the adult, these cells (approximately 2,000 per retina) are clustered along the horizontal striate with the highest density at the area centralis. Amacrine cells within the inner nuclear layer (INL) also are NPY-IR in the adult cell, and these are distributed in a regular array across the entire retinal surface (Hustler et al., in preparation). We have now examined the development of NPY-IR in these two populations of cells. At embryonic day (E) 38 NPY-IR profiles are not present within the GCL or the INL. By E46 such cells begin to appear in a densely packed region of the central retina within the GCL, and by E50 NPY-IR cells within the GCL have extended out to the retinal periphery. At this age their overall distribution is similar to that seen in the adult. NPY-IR cells within the INL do not appear until E50, when they are largely confined to the central retina. About one week later the NPY-IR amacrine cells are first seen in the periphery, with the overall population characterized by a regularly spaced array. During fetal development the number of cells that are NPY-IR was not found to be appreciably greater than in the adult retina. These results indicate that NPY-IR can be utilized during prenatal development to identify specific populations of amacrine and ganglion cells in the cat retina. (Supported by EY03991 from the NEI)
431.17
**RETINAL GANGLION CELLS ARE NOT FRAC TAL IN THE BOX COUNTING MEASURE.** J. Passino and P. Sterling*, Dept. of Neuroscience, Univ. Penn., Phila., PA 19104.

Fractal geometry can be used to characterize patterns that are statistically self-similar, i.e., for which pieces of the pattern, when magnified, resemble the whole. The degree of fractalness is measured by the degree of self-similarity over a certain range of scales. Many physical systems which have been modelled with fractal geometry display a high degree of self-similarity over at least 3 decades of scale. Though retinal ganglion cells also appear fractal, their reported self-similarity spans at most 1 decade of scale and has not been rigorously evaluated.

Images of HRP-filled retinal ganglion cells (from this and other labs) were digitized, then "skeletonized", and overlaid with a square grid of mesh size d. The number of boxes intersected by the skeleton was counted. In a plot of log(boxes intersected) vs. log(d), fractalness is assessed by the degree of linearity over a designated portion of the abscissa. As a control, "box counting" was applied to patterns generated to express self-similarity over specified range of scales. Also, we manipulated the spatial structure of neural and control skeletons according to various randomizing algorithms and observed the effects on the log-log plots.

The log-log plots for the controls have linear regions spanning more than one decade of scale. However, for the neural patterns there are no significant linear portion; instead they are truly sigmoidal: not fractal. We then established an operational criterion: we designated a pattern "fractal" if it arbitrarily deformed the pattern made its log-log plot less linear. The plots for control patterns lost most of their linearity, but the plots for neural patterns were unaltered by the deformation. Thus we conclude that, in the box counting measure, ganglion cells are not measurably fractal. Supported by EY08124.

431.19
**TRANS-ACPD EVOKE S INCREASES IN INTRACELLULAR FREE CALCIUM CONCENTRATION IN ISOLATED RETINAL GIALL (MULLER) CELLS. S.A. Krinsten* and R.F. Miller. Department of Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

Electrophysiological studies have demonstrated that retinal ganglion cells do not respond to application of ionotropic glutamate receptor agonists (NMDA, quisqualate acid, kainic acid). Rather, these cells respond only to application of glutamate with an inward current generated by an electrogenic glutamate uptake mechanism (Hosobuchi and Miller, 1986, Soc. Neurosci. Abstr. 12: 169; Brew and Atwell, 1987, Nature 327: 707-709). The calcium imaging studies that we report here revealed that the metabotropic glutamate receptor agonist, 13,15R-1-aminoacycloptane-1,3-dicarboxylic acid (trans-ACPD) evokes increases in the intracellular free calcium concentration ([Ca²⁺]i) of isolated Muller cells.

Muller cells were acutely isolated from the retina of the larval tiger salamander and loaded with the calcium sensitive dye Fura-2 AM. A commercially available imaging program was used to analyze each calcium change in [Ca²⁺]i evoked by bath application of trans-ACPD (50-200 μM) in calcium-free Amphibian Ringer (2 mM EGTA). Nine of eleven Muller cells tested responded to the application of this metabolite with an increase in [Ca²⁺]i. In six of the responsive cells the increase in [Ca²⁺]i occurred later in the endothelial cell of the cell. In one cell the [Ca²⁺]i of the region of interest was lower at rest that in the other regions of the cell, and the response to trans-ACPD in the nucleus occurred later than the other regions, and was of a larger amplitude. Further experiments include the pharmacological characterization of these responses to determine if they are due to specific activation of the metabotropic glutamate receptor and if glutamate itself also evokes increases in [Ca²⁺]i in Muller cells. Supported by NIH grant EY03014 to R.F. M.

431.20
**TRYPSIN BLUE STAINING ON RETINAL GANGLION CELL OF RAT IN- SULTED IN EXPERIMENTAL HIGH INTRACRANIAL PRESSURE. ZH, Lin, QL, Liu, PB, Ju, ZJ, SHI, and XY, LOP.* Dept. of Neurobiology, Human Medical University, Changsha, Hunan 410078 P.R.C.

We have continued the study on damaged retinal ganglion cell (RGC) resulting from ischemia-reperfusion (I/R) in experimental high intracranial pressure. The experimental high intracranial model was used as before (Lin et al., Soc. Neurosci. Abstr. 15: 1507 91). In 10 albino rats, two eyes of each rat were enucleated and fresh whole mounted retina were prepared for disc-excision test. Trypsin blue (TB) was used as a marker to determine the viability of RGC and was applied according to the method of Taylor and Hant (Brit J. Ophthal. 65:815 91). The nuclei and perikaryon of RGC, which lost its viability, were full of TB substance. For statistics, the stained RGCs within the four quadrants of each retina were counted at a microscopic magnification of 200X with the help of fixing a scored reticle on the slide. The area of whole retina and its four quadrants were digitized respectively with an image analysis system (PC Vision Plus). The data were analyzed for statistical significance by Student's t-test. Using a computer. At comparing these two kinds of rat, far more RGCs of the FR retina were stained than those of the SI (simple ischemia) retina (p<0.001). The results indicated that the diminution of RGCs' overall survival in FR retina with increasing intracranial pressure has been removed. The increased permeability of membrane of RGCs may be related to the attack of the free radical produced in the reperrufying periods as reported by us. Supported by NSFC 3870021 to ZH, Lin.

432.1
**OCCIPITOTEMPORAL AND PARAHIPPOCAMPAL GYRUS PROJECTIONS TO THE BASIS PONTIS IN RHESUS MONKEY. L.D. Schmolesky, D. Purdy, Massachusetts General Hospital, Boston, MA, and ENRR Veterans General Hospital, Bedford, MA, 02102.

We used tritiated amino acids to study projections to the basilar pons from parietal cortices in thirteen rhesus monkeys to determine how connectional and functional heterogeneity of these regions are reflected in corticopontine circuitry. Labelled fibers travelled with every major associative corticocortical fascicles as they arched over the dome of the lateral geniculate nucleus and entered the cerebral peduncle. Pontine projections were defined from area 19 at the medial temporal part of the posterior parahippocampal gyrus, and from the caudal part of the parahippocampal gyrus (area TF). No pontine projections arose from the ventral prefrontal gyrus or from the medial temporal part. Many fiber systems that in the pons were observed in the dorsolateral and lateral nuclei, and the lateral part of the peripendicular nuclei. Medial convexity projections produced more extensive nocosuaral labelling, as well as terminations in the extreme dorsolateral nucleus. Dorsal prefrontal projections had additional terminations in the ventral pontine nucleus. Posterior parahippocampal gyrus projections had discrete label in the lateral and dorsolateral nuclei. These results suggest that the dorsal visual stream communicate with the pons whereas the ventral visual stream does not. The posterior parahippocampal gyrus implicated in visual spatial memory also sends efferents to the pons. These anatomical results have implications for cerebellar function. (Supported in part by NIH 16841 and the Veterans Administration.)

432.2

The neurons in the deep lamina of the superior colliculus (SC) send descending efferents to a variety of regions of brainstem and spinal cord involved in orienting the eyes, head and limbs indicative of important role of the collicular neurons for "visually guided orientation behavior." In an attempt to detail the corticofugal and corticocortical projections from the visual cortex in the rat, we injected the tracers such as biotinyl or WGA-HRP into visual cortex of Long Evans rat and observed the terminal distributions of the corticofugal efferents. After injection of the tracer into area 17, numerous labeled axons and terminals were observed in the superficial laminas of SC. In this case, only a few, if any, labelings were found in the caudate putamen. On the other hand, when the tracer was injected into area 18a, a number of labelings were found in the dorsocaudal region of caudate putamen as well as in the deep lamina of SC. The present results suggests that these corticofugal projections are controlling the visually guided orientation behavior as those found in the cat's corticostriatal and corticocortical projections from the lateral suprasylvian visual area (Torita et al., Neuroscience Res. 10: 149-155, 1991).
423.3 CORtical DEACTIVATION DISRUPTS MULTISENSORY INTEGRATION. L.E. Wilkinson*, M.A. Meredith*, and B.E. Stein†. Departments of Psychology, Anatomy, and Physiology, Medical College of Virginia, Richmond, VA, 23298.

The cortex of the anterior ectosylvian sulcus (AES) contains unimodal visual, somatosensory and auditory neurons whose projections converge, along with those from the lateral suprasylvian cortex (LS), onto multisensory superior colliculus (SC) neurons. These SC neurons are believed to play important roles in attentive and orienting behaviors. In the present experiments we sought to determine the effect of reversibly deactivating these cortices on such behavior. The testing apparatus consisted of a semi-circular array of paired LEDs and speakers at 15° intervals. Two cats were trained to look at each with a near-threshold visual stimulus, regardless of location, for a food reward. The auditory stimulus was presented at the same site as the visual stimulus, or 45° lateral or medial to that site. In normative testing, animals showed a multiplicative enhancement of their responses to visual stimuli when an auditory stimulus was presented spatially coincident with the visual stimulus. Conversely, they showed dramatic inhibition of responses to the visual stimulus when the auditory stimulus was 45° disparate to it. During testing, a cortical area (either AES, LS, AI/All, or striate cortex) was deactivated with lidocaine through indwelling carotid. Deactivation of AES disrupted multisensory enhancement and multisensory depression at all eccentricities tested. However, deactivation of the other cortical areas tested, and/or saline controls in AES had no effect on these multisensory processes. Supported by NIH grant NS 22543.

423.5 CORTICOSTRIATAL AND CORTICOTOPIC PROJECTIONS FROM THE FRONTAL EYE FIELDS OF THE CAT. M.T. Thomson*, B.E. Stein and J.G. McHaffie*. Department of Physiology, Medical College of Virginia, Richmond, VA, 23298.

The relationship between the frontal eye fields (FEF) and the striatum (ST) of the cat are poorly understood. The present experiments were an attempt to determine how regions surrounding the presylvian (FEF) region project to the ST. Injections of WGA-HRP made into both cortical regions resulted in dense labeling in the ST and the SC. In the ST, anterograde labeling was found bilaterally (with a leftward predominance) in the caudal part of the SC, in the head of the caudate nucleus (AP +17.5 to +14.0); only presylvian injections resulted in labeling in the putamen, where it was restricted to the caudal aspect. Presylvian injections resulted in label distributed dorsally whereas cruciate injections produced label ventromedially. These same cortical injections also resulted in label restricted to the deep lamina of the ipsilateral SC. After injections of WGA-HRP into ST, numerous labeled corticostriatal neurons were observed. In the presylvian, they were found in lamina III and the upper aspects of lamina V while in the cruciate, they were located in lamina III, upper aspects of lamina V, and lamina VI. In both regions, corticostriatal neurons were observed only in lamina V. These data are consistent with the idea that, in addition to a direct influence on deep laminae SC neurons, the FEF may modulate tectospinal neurons indirectly via the ST and substantia nigra. Supported by NEI grant EY05554.


Visual, auditory and somatosensory inputs from anterior ectosylvian (AES) and lateral suprasylvian (LS) cortices converge onto multisensory superior colliculus (SC) neurons (Wallace et al., 1991). In this study we sought to determine how these corticostriatal inputs influence the response properties of SC neurons. Reversible deactivation (cooling) of a single modality-specific cortical region (i.e., SIV-somatosensory, FEF-auditory, and LV-visual) was found to depress the corresponding unimodal response of a multisensory SC neuron (i.e., cooling SIV depressed somatosensory responses of a visual-somatosensory neuron). In many cases this deactivation also resulted in a proportionate decrease in the integrative properties produced by presenting two different sensory stimuli simultaneously. However, in certain instances deactivating a single cortical region altered multisensory integration in SC neurons in ways not readily predictable from its effects on unimodal responses. For example, in some neurons deactivation of SIV decreased visual-somatosensory integration to a far greater extent than that expected based on the slight depression of the unimodal somatosensory response. In select cases a seemingly paradoxical effect was observed when multiple cortices (i.e., LS, AES and SIV) were deactivated simultaneously: unimodal visual and somatosensory responses were depressed, but the magnitude of the multisensory interaction was dramatically increased (from 84 to 495%). This is likely due to the principle of "inverse effectiveness," wherein the combination of weakly effective unimodal stimuli produce disproportionately high enhancements of one another's effect on SC activity. These data demonstrate the importance of the different unimodal cortices in maintaining the integrity of multisensory integration in the SC. Supported by NIH grants NS 08902 and NS 22543.

423.7 "Extrastrate" Visual Pathways in the Pigeon: Descending Projections upon the Optic Tectum. Harvey J. Karten*, Kevin Cox and Taka Shimizu. Deps. Neuronsciences, University of California San Diego, La Jolla, CA 92039 and Dept. Psychology, University of South Florida, Tampa, FL 33620.

Two major visual pathways to the forebrain have been identified in birds: 1) A topotectal pathway, the retina-tecto-estomo-esto-ecoc system similar to the extrastriate pathways through inferior pulvinar of mammal; and 2) A retinotecto-talamo-ecto-striatal system similar to the geniculostriate system of mammals. Two major descending pathways from tectopallium upon the optic tectum in pigeon have been identified. These arise from: a) The waltz, (Karten et al., 1975, 1977, 1982), and b) The architecturus (Zierer and Karten, 1975), a patterned target of the roundo-ectostriatal pathway. Chorera toxin B (CTB) was used to identify the locus of origin within the architecturus of projections upon the tectum, and to then characterize the pattern of termination of these projections within the tectum. Telencephalic Origins of Tectal Afferents: Following injections of CTB limited to the optic tectum, without spread to underlying ventricle or subnuclear, magnocellular labeled cells in the telencephalon were confined to two regions: a) A deep restricted zone within the ventromedial portion of the archistriatum medullare (Ziel); and, b) The hyperstriatum accessorius of the wall, and adjacent cortical lateral portion. Projections of the Architecturatum upon the Optic Tectum: Injection of CTB in the AV resulted in labeling in the tract occurring mesencephalons with terminations in Layers 10-13 of the ipsilateral optic tectum. Projections of the waltz termini to more superficial laminae of the tectum. The pattern of differential distribution of waltz vs. architectural projections resembles the differential pattern of projections of corticocellular connections in mammals arising from the striate vs. extrastriate cortices, respectively. Supported by NS-25450-06 and ONR N00014-88-K-0054 to HJK.
432.9 LINEARITY AND DYNAMICS OF THE RETINAL SLIP CODE IN TURTLE ACCESSORY OPTIC SYSTEM NEURONS. A.P. Rosenblatt and M. Ariel. Dept. of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260, USA.

The basic optic nucleus (BON), the major retinal recipient area of the retinl accessory optic system, contains direction-sensitive (DS) cells that respond to full-field, textureless visual stimuli. It is thought that these neurons provide a retinal slip signal that drives the optokinetic reflex. The purpose of this study was to quantitatively investigate how retinal slip is encoded in these neurons using linear system theory.

Single unit responses of BON neurons were characterized using whole-field visual stimuli projected directly onto retinas of isolated turtle brains. Direction-tuning curves were obtained either by using constant velocity stimulation (linear sweeps) or by presenting a constant speed stimulus that moved along a circular path (circular sweeps). The hypothesis that stimulus velocity is encoded along a single linear axis was first tested by fitting direction-tuning curves to cosines. Second, the direction-tuning derived from circular sweeps was compared to the sum of responses of two orthogonally oriented stimuli, each of which followed a linear path with sinusoidally modulated velocity (sinusoidal sweeps). Finally, linear sweeps were applied along the axis with maximal DS modulation at different speeds to directly test the cosine rule. The tuning of these cells obeys a cosine rule to a first approximation, with the addition of a non-DS component.

To assess the dynamic responses of BON neurons, sinusoidal sweeps were applied along the axis with maximal modulation at several frequencies. The resulting discharge rates were fit to rectified sinusoids to determine gain and phase. Sinusoidal modulation persisted at frequencies to 1 Hz with peak speeds below 15°/s. BON neurons exhibit DS responses at a broad range of stimulus velocities, yet the range of movement for which these cells exhibit somewhat linear responses seems to be restricted to slow velocities.

Supported by EY05978 and M00815.

432.11 CENTRIFUGAL PROJECTIONS TO THE RETINA IN THE TURTLE Pseudemys scripta elegans. D. Zhang and W.D. Eldred*. Dept. of Biology, Boston University, Boston, MA 02215.

The existence of centrifugal modulation of different fibers to the retina from the brain has been demonstrated in many vertebrate species, including the turtle. However, little detailed information is known regarding the number of cells involved and the location of the cells in the brain which give rise to these centrifugal fibers. To investigate this question, we used intracranial injections of cholera toxin B to retrogradely label the cell bodies in the brain which project to the eye. The labeling of cell bodies in the brain were visualized using an antibody directed against cholera toxin B. Approximately 40 centrifugal cell bodies were found on each side of the brain. The locations of these neurons were not confined to specific nuclei within the brain. The majority of cell bodies were concentrated in the nucleus of isthmi paraventricularis. This nucleus is homologous to the isthmo-optic nucleus in birds, which also gives rise to centrifugal fibers to the retina. Additional cells were observed in the nucleus reticulata OPT, the lateral geniculate, and in the lateral wing of the dorsal raphe nucleus in the reticular formation of the mesencephalon. The localization of these cell bodies within the brain will provide the basis for future studies of their role in visual processing. This research supported by EY04785 to WDE.


Woodson et al. (Soc. Neurosci. Abstr.'90) 16: 1313 reported three distinct morphological types of presumptive centrifugal terminals (PTs) in the pigeon retina. Subsequent investigation revealed that: (i) the isthmo-optic nucleus (ION) proper and the ectopic cells each give rise to different types of centrifugal PT endings; (ii) high density areas were observed in the inferior division of the retina, and on both sides of the pecten; (iii) overlap of different types of centrifugal PTs was observed in the optic nerves.

The distribution and morphology of centrifugal endings of 21 day old chicks were examined following injections of 1% cholera toxin B into ION and the surrounding tegmentum. As in pigeons, centrifugals are found mainly in the inferior retina. One type has a thin axon with small varicosities (~ 1 μm in diameter) consisting of 5-10 smaller varicosities (~ 1 μm in diameter); (ii) the presence of two types of arborizations in the intratretinal retina, and only the thin widely arborizing type in the ipsilateral retina. The results suggest a transient developmental stage or a species difference in the morphology of centrifugal PT arborizations. Moreover, we suggest that the thin and widely arborizing ipsilateral centrifugal projections in the chick retina arise from the ectopic cell region.

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432.13 TECTAL VISUAL RESPONSES ARE MODULATED BY ISTMIC NEURONS IN PIGEONS. Y.-C. Wang, S.-F. Wang and B.J. Frosch. Departments of Physiology and Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Anatomical studies in pigeons have shown that both nucleus isthmi paraventricularis (IPc) and nucleus isthmi magnocellularis (Imc) receive input from, and feedback to, the optic tectum. However, the functional effects of these neural circuits on the tectal cells are still unknown. The present study evaluates the roles of Ipc and Imc in modulating the visual response properties of tectal neurons. In Ketamine/Rompun anaesthetized pigeons, Ipc or Imc was reversibly blocked by lidocaine (2%, 5-9%) or excited by NMDA (30 mM, 5-9%) while quantifying tectal neurons (n=65) with moving visual stimuli. Single cell recordings showed that 80% of the tectal neurons were almost totally inhibited after injection of lidocaine into the region of Imc having receptive fields (RFs) overlapping the tectal RFs. However, 68% of the tectal neurons were unchanged when the same drug was applied to Ipc under the same conditions. In contrast, administration of NMDA into Ipc dramatically reduced the responses of 71% of tectal cells, while NMDA applied to Imc had no effect. These feedback effects are tightly related to the topographic relation between RFs in nucleus isthmi (Ipc and Imc) and optic tectum. Removal of Ipc-tectal or Imc-tectal projections (Ipc-Imc pathway) significantly reduced the excitability of tectal neurons whose RFs were located within the isthmic RFs, otherwise, there was no effect. These results suggest that both nucleus isthmi (Ipc and Imc) play a differential feedback role in modulation of visual processing in the avian optic tectum. Supported by NSERC OGP0000353 and SED117027.
432.3 COULOMOTOR RESPONSES TO PERCEPTUALLY COHESIVE AND NON-
COHERENT PLAIDS: KEI DEPPEK,* GH SPERLE AND TL ALTREY. The Salk Institute, La Jolla, CA 92037.

When two moving gratings are superimposed, they can be perceived as either a single coherently moving "plaid pattern" or as two component gratings sliding non-
coherently across one another. Since visual motion signals are known to have strong influence over oculomotor activity, we sought to determine whether there is correspondence between involuntary eye movements elicited by moving plaids and judgments of motion coherence. Specifically, we asked whether direction and speed of eye motion in human subjects were independent of perceptual coherence or, alternatively, contained component motion when non-coherence was reported and pattern motion when coherence was reported.

Plaid patterns were constructed using two heterochromatic (red/green) sinusoidal gratings oriented 87.5° apart. Motion of the plaid was produced by moving only one of the two gratings. Unlike conventional plaids constructed from two moving components, coherant and non-coherent plaids elicited by these novel plaids are each associated with a single direction of motion. Non-coherence is characterized by perceived motion in the direction of the single moving component. By contrast, coherence is associated with perceived motion along the axis of the stationary component. Plaid stimuli were 22° diameter and positioned at the center of gaze.

We used color asymmetries to manipulate perceptual coherence of moving plaid patterns and we recorded accompanying oculomotor activity. Individual gratings within the plaid contained either + (red brighter) or - (green brighter) luminance contrast. When the two heterochromatic gratings contained "symmetric" (+ or -) luminance contrasts, coherent motion was perceived. When the gratings contained "asymmetric" (+) luminance contrasts, non-coherent motion was perceived.

When the plaid was perceived as moving coherently, eye movements were relatively fast and closer to the coherent direction. Conversely, when non-coherent motion was perceived, eye movements were relatively slow and closer to the component direction. These results suggest that the neural mechanisms underlying perceptual motion coherence also participate in the generation of eye movements.

432.9 LESIONS OF LATERAL SUPRASylvIAN CORTEX IN THE CAT REVEAL DEFICITS IN THE PERCEPTION OF GLOBAL MOTION. K. K. RUGKASA* AND T. PASSERON. Center for Vision Science and Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14627.

High incidence of directional selectivity and large receptive fields make neurons in the lateral suprasylvian (LS) cortex of the cat well-suited for the analysis of visual motion. We examined the role of LS cortex in the integration of local motion signals into a global percept. We placed bilateral dorsal retinal lesions in LS cortex and recorded their responses to test the dissociation of motion direction. The targets consisted of dots that were displaced with a constant step size in motion at random from the specified distribution. When the range of the distribution of such displays is limited to about 300 deg, the display appears to move in the direction of the mean. LS lesions produced deficits in the discrimination of opposite directions for large (1 deg) but not small (0.15 deg) step sizes. The spatial limit for the task (Dmax) was also substantially decreased. These results suggest that there is a change in spatial scale of residual motion mechanisms in the absence of LS cortex.

Postoperative motion thresholds were also affected by the addition of dots moving in random directions (directional noise), even at small step sizes. Thus, LS lesions affected the integration of local motion signals in the presence of directional noise, as did lesions in LS cortex. These results are consistent with the importance of LS motion processing in the generation of eye movements (Passeron et al., Unpubl., 1991).

432.11 MOTION DETECTION AND IDENTIFICATION FOR LUMI-

We have measured thresholds for detection and identification of a vertically oriented sinewave grating vignetted by a two-dimensional spatial Gaussian window. The stimulus was presented as a contrast-reversing grating to the left or to the right of fixation on the horizon-
tal meridian and then moved either towards or away from the fovea. The temporal frequency of the contrast-reversing and drifting gratings were identical. The color of the grating was modulated along different directions in color space around an equal energy white, thus leading to different input contrasts for the L- and M-cones. This allowed us to measure the identification contours in cone contrast space (Stromeyer, Eskew, Ryu & Kraooner, ARVO 1991).

We find that contrast sensitivity is particularly impaired for stimuli having no Lcone contrast. When considering the mechanisms that mediate motion detection and identification, we find that under certain spatio-temporal conditions contrast sensitivity is determined by a mechanism that is independent of motion perception. These results are compared to behavioral and physiological data in macaque monkeys.

Supported by NIH grant EY08300.


A random dot pattern (RDP), generated on a computer and moving across the foveal and parafoveal retina (stimulation field 6-8 degrees, 30 s stimulation period), evokes a movement after-effect (MAE) in the direction opposite to the RDP movement, which is consistent for both viewing condition: (a) fixation and (b) eye pursuit movements (head fixed, 0.25 - 8.0 deg/s).

When simultaneously two RDPs ("transparencies") are moved perpendicularly to each other across the stimulation field, two movement patterns are seen, but only one rather uniform MAE is evoked (Verstraten et al., 1990). Its direction D is in between the MAE directions expected for each of the two moving RDPs. D depends on the angular velocities of the two RDPs and on the stimulus conditions (a) or (b). A sensitivity-weighted vector summation predicts the outcome for stimulus condition (a). When, however, with stimulus condition (b) one of the moving RDPs is pursued, D is determined by "retinal" stimulation and the "perceived" motion.

It is concluded that the "weighted vector summation" of the MAE may be attributed to cortical areas (MST, FST 7), where gaze movement signals interact with the visual motion signals. This supported by a F.C.Donders-professorship, University of Utrecht).
434.4 EXPRESSION OF AMPA-SELECTIVE GLUTAMATE RECEPTOR SUBUNIT mRNAs IS RELATED TO SYNAPTIC POTENTIALS IN THE RAT COCHLEAR NUCLEUS. C. Hunter*, T. Yu and R. J. Wenthold, Lab. of Neurochemistry, NIDCD, NIH, Bethesda, MD 20892.

We have previously used in situ hybridization histochemistry to localize AMPA-selective glutamate receptor (GluR) subunit mRNAs in morphologically defined cell types. Selection of GluR subunit antisense oligonucleotides to GluR1-4, 45 nucleotides in length (See Kehnainen et al., Science 249:556-560, 1990) and their sense controls were hybridized to tissue sections of the adult rat cochlea, more in the central part of N, when physiological studies find the most sharply tuned neurons.

The types of GluR subunit mRNAs include: GluR1, GluR2 (layes I and VII), GluR3, GluR4 (layes II-VI), GluR5, GluR6 (layes II-VI), inverted GluR3 (layes I and II), and large ovoid neurons (layes II-VI).

We have used these antisense probes to study the expression of GluR1-4 subunit mRNAs in the rat cochlear nucleus. The expression of GluR1-4 mRNAs is related to the synaptic potentials in the central part of N, where the most sharply tuned neurons are found.

434.5 HOMINGENOUS DISTRIBUTION OF GABA NEURONS ACROSS CAT PRIMARY AUDITORY CORTEX. S. H. Hendry* Dept. of Anatomy & Neurobiology, University of California, Irvine, CA 92717.

The primary auditory area (A1) of cat cerebral cortex contains a map of the cochlea, with each sound frequency represented as a band of dorsocentrally oriented cells (isofrequency bands). In cat AI, tonotopic afferents are unevenly distributed and terminate in broad anteroposteriorly oriented zones that interface isofrequency bands. These tonotopically defined zones are physiologically defined areas in ways suggestive of inhibitory influences that are greater within the latter. To determine if the physiological differences are correlated with different afferent inputs intrinsic to AI, GABA immunoreactive neurons were examined for evidence of an uneven distribution of inhibitory interneurons across AI. Stereological methods were applied to determine the numerical density and proportion of GABA immunoreactive somata in 1 mm-thick sections through 5 hemispheres of 3 normal cats. Mean values of 21,404-22,800 GABA neurons per 1 mm² of cortex, which make up 24-28% of the total neuronal population was calculated for AI. Values for individual layers varied with the greatest density of GABA neurons in layers III and IV and greatest proportion in layer I. Those values for AI in general, and for individual layers did not vary significantly either dorsocentrally across AI (parallel to isofrequency bands) or anteroposteriorly (perpendicular to the bands). These data suggest that regional variations in the total population of GABA neurons do not occur across AI and, thus, cannot account for physiological differences in this area. Evidence for variations in GABA neuronal subpopulations is currently under investigation. Supported by DC 00540.
434.7
LONG-TERM EFFECTS OF COCHLEAR AND MIDDLE EAR LESIONS ON SYNAPTIC TRANSMITTER RELEASE IN THE COCHLEAR NUCLEUS. C.G. Benson* and S.J. Potashner, Department of Anatomy, University of Connecticut Health Center, Farmington, CT, 06030.
To determine if cochlear activity affects the regulation of synaptic function in the cochlear nucleus (CN), we measured the effects of unilateral cochlear ablation and unilateral osseous disarticulation on the activity of glutamatergic or aspartagertic and glycinegic synaptic endings, in the CN of adult guinea pigs. Guinea pigs were anesthetized and either the left cochlea was destroyed mechanically or the left middle ear ossicles were disarticulated. Two days later, 5-9 weeks after the uptake and release of [3H]-aspartate ([3H]-D-ASP) and [3H]-glycine were measured bilaterally in dissected segments of the CN, in vitro. Two days and 8 weeks after cochlear ablation, [3H]-D-ASP uptake and release were reduced ipsilaterally in all segments. However, by 16 weeks after the lesion these measures had recovered and were no longer significantly different from controls. There were no significant changes in the uptake and release of [3H]-glycine in the CN after cochlear ablation. Sixteen weeks after unilateral osseous disarticulation there were significant decreases in the uptake and release of [3H]-D-ASP bilaterally in the CN. These findings suggest that chronic changes in cochlear activity can lead to changes in synaptic release in the CN. (Supported by DC00199 from NIH NICD)

434.8
Evidence suggests that glutamate, aspartate, GABA, and glycine may be transmitters in the cochlear nucleus. In this study, we measured the release of [3H]-D-aspartate, [3H]-GABA and [3H]-glycine from several other brain stem auditory nuclei to determine if they too might contain synaptic endings that use these amino acids as transmitters. After excision, the brain stem was cut transversely into 500 μm sections from which auditory nuclei were punched. The accuracy of punching was confirmed by histological analysis. Punches were incubated with [3H]-D-aspartate and [3H]-GABA or [3H]-glycine before the release of radioactivity was assessed in a superfusion system. Electrical stimulation evoked the Ca2+-dependent release of D-aspartate from each punched nucleus, namely the LSO, MSO, MNTB, VNLL, and the central nucleus of the IC (ICc). Ca2+-dependent release of GABA was observed from the MNTB, VNLL, and ICc while release of glycine was observed from the LSO and MSO. These findings are consistent with the hypothesis that these nuclei contain glutamatergic or aspartagertic synaptic endings. They also suggest that some of these nuclei may contain GABAergic and glycinegic synaptic endings. (Supported by DC00199 from NIH NICD)

434.9
PLASTICITY OF CALBINDIN IMMUNOREACTIVITY IN THE MATURE GERBIL SUPERIOR OLIVARY COMPLEX WITH ALTERED AUDITORY EXPERIENCE. M.D. McGinn*, J.R. Schwartz, and P.R. Eager*. Dept. of Otologyngology, UC-Davis, Davis, CA 95616 and Sect. of Otologyngology, Yale University School of Medicine, New Haven, CT 06510 U.S.A.
Calbindin D-28k immunoreactivity (CaBP) showed consistent and distinctly different patterns in the MNTB and LSO which varied with auditory experience. Animals were colony reared(COL), exposed to 3 weeks of intermittent (30 min on/off) high (HF)(80dB) or low frequency (LF)(74dB) noise, or to low sound (LIG)(ear canal ligation). The MNTB showed the most intense CaBP+ cells in all conditions. HF animals showed the largest number and most intense CaBP+ cells and the fewest light CaBP+ cells. OL animals showed a greater proportion of light CaBP+ cells; COL animals were similar. LIG animals showed the highest proportion of light CaBP+ cells.
The LSO showed the greatest variation with the different treatment conditions. HF animals showed a densely stained neuropil in the medial limb. Moderately stained cells were found throughout, but some cells in the medial limb were darker. LF animals showed many moderately stained cells in the lateral limb, but fewer in the medial limb. Medial limb CaBP+ cells stained more darkly, but less intensely than in HF animals. The medial limb neuropil stained more densely than the lateral limb, but less darkly than in HF animals. LIG animals showed many lightly stained cells throughout the LSO in a uniformly stained neuropil comparable in intensity to that in the medial limb of LF animals. (Supported by NIH/NICD grants DC00132 and DC00057).

434.10
PLASTICITY OF CALCIMUM BINDING PROTEIN AND GABA IMMUNOREACTIVITY IN THE ADULT GERBIL COCHLEAR NUCLEUS WITH ALTERED AUDITORY EXPERIENCE ORAL. W.E. Ose*, P.R. Eager and M.D. McGinn*. Sect. of Otologyngology, Yale University School of Medicine, New Haven, CT 06510 and Dept. of Otologyngology, UC-Davis, Davis, CA 95616 U.S.A.
Calbindin D-28k (CaBP), parvalbumin (PV) and GABA (GABA+) immunoreactivity showed patterns in the cochlear nucleus (CN) which varied with auditory experience. Animals were colony reared(COL), exposed to 3 weeks of intermittent (30 min on/off) high (HF)(80dB) or low frequency (LF)(74dB) noise, or to low sound (LIG)(ear canal ligation). The LSO showed large numbers of intensely stained GABA+ puncta in the DCN and FCN which were not seen in HF, LG or OL animals. The intensity and number of CaBP+ dendrites in the DCN molecular layer decreased from LIG to HF to LF animals. COL & LF animals were similar. In octopus cell regions the darkest CaBP+ somata were in HF. The greatest extent of dendritic staining was in LIG animals which also showed the greatest number and size of CaBP+ puncta. PV+ somata in the DCN molecular layer decreased in intensity from HF, to LF to LIG. Changes in CaBP+ and PV+ in the CN were less marked than the changes observed elsewhere in the auditory brainstem. In the superior colliculus LF animals showed an almost complete absence of PV+ stained cells and dendrites compared to HF animals. The observations are consistent with the hypothesis that a strongly driven auditory system protects neurons from overstimulation by an upregulation of inhibitory inputs. (Supported by NIH/NICD grants DC00132 and DC00057).

434.11
QUANATIVE EVIDENCE THAT COCHLEAR ROOT NEURONS ARE NOT CHOLINERGIC. W.Yao and D.A. Godfrey*. Dept. of Otolaryngology, Med. Col. of Ohio, Toledo, OH 43699.
Cochlear root neurons (CRN) are distinct morphologically from globular bushy cells in retinal auditory nerve root (Merchant et al., 1988). CRN do not immunoreact for glycine or GABA (Osen et al., 1981). Our preparations have shown immunoreactivity of CRN for choline acetytransferase (CHAT) which, despite their very weak histochemical reaction for acetylcholinesterase, suggests that they might be cholinergic. To provide evidence concerning this, we used autoradiographic techniques to measure CHAT activities (μmol/kg/min) both of samples containing CRN and of adjacent samples not containing CRN from 5 rats. Mean (±SD) (μmol/kg/min) CHAT activity of CRN-containing samples was 2.29±0.46 (n=5) while that of the non-CRN samples was 31±18.13. By measuring proportions of the samples occupied by CRN cytoplasm, we estimated the CHAT activity of CRN to be 2.29±0.46(n=5). By contrast, the CHAT activity for samples of the facial nucleus, which contains cholinergic motoneurons, was 482±11.84(n=5), and that for samples of the facial motor root was 6074±1397(n=4). Thus, CRN are not likely to be cholinergic neurones. However, the presence of CHAT activity in some samples suggests that they might receive some cholinergic innervation. (Supported by NIH grant DC00172)

434.12
EFFECTS OF CHOLINERGIC AGONISTS AND ANTAGONISTS ON SPONTANEOUS ACTIVITY OF RAT DORSAL COCHLEAR NUCLEUS NEURONS: IN VITRO STUDIES. L. Ose, J. H. J. Wallier* and D. A. Godfrey*, Dept. of Otolaryngology and Neurological Surgery*, Medical College of Ohio, Toledo, OH 43699.
Extracellular recordings from rat brain stem slices tested the effects of bath application of cholinergics and antagonists on cochlear nucleus neuronal activity. Recordings were made from 54 neurons in 3 slices from 28 rats. Of neurons tested, 89% showed increased firing in response to 10 μM carbachol (mean increase = 249%, n = 18), 63% showed increased firing to 10 μM muscarine (mean increase = 529%, n = 35); and 50% showed increased firing to 200 μM carbachol (mean increase = 103%, n = 12). Only 3% and 25% of the neurons showed decreased firing to muscarine and nicotine, respectively. The excitatory effect of carbachol was eliminated by atropine (1 μM, n = 3), but not by d-tubocurarine (2 μM, n = 2). Excitatory effects of muscarine were blocked by 1 μM atropine (n = 6), but not by selective antagonists of muscarinic receptor subtypes: pirenzepine (1-5 μM, M1 receptor, n = 3), galantamine (10-20 μM, M2 receptor, n = 3) or p-fluorohexahydro-silidindol (1-2 μM, M2 receptor, n = 2). We also found that 40% of the neurons tested showed an excitatory response to 50-100 μM serotonin (mean increase = 190%, n = 20, 60% were unaffected). (Supported by NIH grant DC00172)
435.6 FUNCTIONAL ORGANIZATION OF ACOUSTIC AND VOCALIZATION AREAS OF THE BAT CENTRAL NERVOUS SYSTEM REVEALED BY HIGH RESOLUTION AUTORADIOGRAPHIC IMAGING OF \(^{3}H\)-2-DEXTROGLUCOSE UPTAKE. G.E. Duncan, W.E. Stumpf, and W.W. Holloway, Dept. of Cell Biology and Anatomy, Univ. North Carolina, Chapel Hill, NC 27599

Brain activity patterns associated with the generation and processing of vocalization signals in the bat Pteronotus p. parnellii were studied by high resolution autoradiographic imaging of \(^{3}H\)-2-deoxyglucose uptake. Bats were injected i.p. with \(^{2}D\)-glucose and restrained in a foam holder or allowed to fly in the laboratory. Bats were killed 20 min after injection of \(^{3}H\)-2-deoxyglucose. In the flying bats, marked alterations in patterns of 2-DG uptake occurred in brainstem and forebrain regions. Regions activated during flight included the nucleus ambiguus, inferior colliculus, superior colliculus, medial geniculate, auditory cortex, and nucleus trascus. The results help to define the functional organization of brain regions involved in vocalization and processing of biosocial signals.
435.7 CYTOARCHITECTURE AND HISTOCHEMICAL ORGANIZATION OF ODONTOCETE WHALE AUDITORY BRAINSTEM. V.E. O'Neill*, M.E. Settel*, S.M. Baber*. Dept. of Physiology' and Neurobiology and Anatomy*, Univ. of Rochester School of Medicine, Rochester, NY 14642.

Relatively little is known about the anatomy of the central auditory systems of echolocating whales. We studied the brainstem of the beluga whale and bottlenose dolphin. The auditory nuclei: the medial geniculate, acoustic, cholinesterase (AChE), and antibodies to Calbindin D-28K (cab) and parvalbumin (pv) were examined to localize and characterize the structures of the auditory brainstem. Comparisons were made to material from bats, rodents, human and non-human primates. In the beluga whale, an extremely large (5 mm diameter) auditory nerve leads into a large cochlear nucleus (CN). Many cab(+) and pv(+) cells were scattered throughout the anteroventral CN. Cells in the prominent lateral superior olive were cab(-) and pv(+), as in other mammals. The medial nucleus of the trapezoid body (MTNB) was both cab and pv positive. Medalial to and apparently associated with the MTNB, there is an unusual disconcentrally oriented, sickle-shaped sheet of cells, embedded in a band of AChE(+) fibers. These cells may belong to the medial oculocochlear bundle system. Although the general features are consistent with those in other mammals, certain aspects appear unique to the whale, and may reflect adaptations to echolocation in an aquatic environment. (Supported by NIH NS-22511 and DC-0267)

435.9 PARVALBUMIN IMMUNOCYTOCHEMISTRY DELINEATES PRIMARY AUDITORY NEOCORTEX. C.B. Smelser and N.T. McMullican*. Department of Anatomy, University of Arizona College of Medicine, Tucson, AZ 85724.

Electrophysiological and anatomical studies of rabbit auditory neocortex have revealed a large primary field (AI) characterized by a distinct cytoarchitecture: a cell-dense lamina III/IV and a broad cell-sparse lamina V. We report that monoclonal antibodies to parvalbumin (PV), a calcium-binding protein present in GABA-ergic local circuit neurons, precisely delineate the AI cytoarchitecturally defined AI PV immunocytochemistry (PV; SWANT, 1:10K) was performed on 100 mm thick sections obtained from young rabbits. Serial coronal sections through the entire temporal pole were processed and processed and the third section was Nissl stained with acetylcholine esterase, PV yields an extraordinary Golgi-like dendritic (and, in some cases, axonal) labeling of nonpyramidal cells in all cortical layers. The dorsal boundary of AI is demarcated by an abrupt increase in the number of PV-positive nonpyramidal cells and dense terminal labeling within lamina III/IV. Lamina II and upper parts of III are populated by nonpyramidal cells with small somata and local cell bodies of the bifurcated, bipolar and stellate variety. Lamina III/IV is characterized by a dense population of large spiny nonpyramidal cells with bifurcated dendritic domains and tangentially-oriented local axonal plexus. Basket type axonal terminations outline pyramidal cell somata and contribute to the dense terminal labeling within III/IV. Cell-sparse lamina V contains large nonpyramidal cells remarkable for their tangentially-oriented dendritic fields. Lamina VI can be partitioned into sublamina Vla and Vlb. Lightly labeled pyramidal neurons compose Vla while a diverse population of nonpyramidal cells occupies Vlb. The ventral border of AI stands in stark contrast to the near absence of PV labeling in perirhinal cortex (Supported by NIH, Deafness Research and Whitelab Foundations).

435.10 DIFFERENTIAL CORTICAL PROJECTIONS FROM SUBNUCLEI OF MONKEY MEDIAL GENICULATE COMPLEX REVEALED BY ANTEROGRADE TRANSPORT OF PHA-L. T. Hashikawa*, H. Hidaka and F.G. Jones. Laboratory for Neural Systems, F.R.P., RIKEN, Wako 351-01, Japan, and Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Cortical auditory fields have been shown to receive their thalamocortical afferents from specific subdivisions of the medial geniculate body (MGB). In the present study, in order to examine how fibers originating from each MGB subdivision contribute to the projections of Phallus vulgaris leucoagglutinin (PHA-L) was iontophotographically injected under physiological control in limited regions of the MGB in Japanese monkeys, Macaca fascicularis.

Fibers from the ventral nucleus formed several terminal plumes in a patch-like fashion in the primary auditory cortex (AI), mainly in middle cortical layers. When measured in tangential sections, the length of the plume was 500-1000 μm in width. A main patch was surrounded by "satellite" patches and their projection were not to A1. The size of the main patches was twice as wide as those in AI even with smaller injections. Fibers from the magnocellular nucleus had rather few terminal branches in middle cortical layers as well as in layer I, but they innervated extremely wide areas, both in AI and surrounding auditory areas by means of collaterals of single axons. Formation of discontinuous terminal patches suggests the presence of unitary modular structures in the auditory cortices based on thalamocortical connections.


The crossed and the uncrossed projections from the lateral superior olive (LSO) to the inferior colliculus (IC) are distinct in all respects. The correlation between the birthdates of the LSO neurons and the laterality of their projections was studied in the rat by double-labeling techniques using 5-bromodeoxyuridine (BrdU), the thymidine analoge, and Fluorogold (FG), a retrograde fluorescent tracer. BrdU was given to a pregnant rat on each day throughout the LSO generation period (E12-E16). In the progeny rats as adults, FG was injected into the IC unilaterally to differentiate the crossed and the uncrossed projection neurons. The results indicate that the crossed projection neurons were mainly produced on day E13, whereas the uncrossed projection neurons generated from day E14 to E16 with peak production on day E15. Thus, one of the factors initiating the target laterality of the projections is the temporal order of neurogenesis.
435.1
EXCITOTOXIC PARABRACHIAL NUCLEUS LESIONS DISRUPT CONDITIONED TASTE AVERSION, CONDITIONED ODOR AVERSION, AND SODIUM APPETITE IN RATS. C. Scalara*, P. Grigson, T. Shimura, S. Ryall, and R. Norgrove. College of Medicine, Penn State Univ., Hershey, PA 17033

Previous studies have demonstrated that electrolytic lesions in the parabrachial nucleus (PBN) disrupt both conditioned taste aversions and salt appetite in rats. In order to assess the contributions of neurons and fibers of passage to these effects, we made electrolytically guided bilateral injections of ibotenic acid into the PBN of 10 rats (IBO, 0.2 ul; 20 ug/jl). Six other rats received the same volume of vehicle in the PBN (PBS, pH=7.4) and 4 animals served as non-surgical controls. Following overnight fluid deprivation, rats were given 15 min access to 0.3 M saline and immediately injected with IBO (1.5 mg/kg, 0.15 M). Three saline-IBO pairs were spaced 3 days apart. All non-injected rats rejected saline following a single pairing with IBO. Even after 3 pairings, however, the IBO rats failed to reject saline.

Subsequently, it was found that some rats were dispelled with 10 micrograms of homoside (7.0 mg/ml) and given simultaneous access to 0.5 M NaCl and water. The IBO rats increased intake of NaCl, while the other groups consumed at least 3 times control levels. In a second set of rats, using a higher concentration of IBO (0.3 M), IBO lesions of the PBN prevented acquisition of both an saline taste aversion and an almost odor aversion. Finally, the lesioned rats exhibited an apparent lack of neophobia in 2 situations -- when first presented with saline and when put on a novel, Na-free diet. Supported by DC-00240, DC-00047, MH-43787, MH-00653.

435.14
PROJECTIONS TO THE COCHLEAR NUCLEUS FROM PRINCIPAL CELLS IN THE MEDIAL NUCLEUS OF THE COCHLEAR-TYMPANIC TRACT IN THE HAMSTER. R. R. Scheinfeld* and N. B. Cant. Dept. of Neurobiology, Duke Univ. Medical Center, Durham, NC 27710

The superior olivary complex is a major source of descending projections to the cochlear nucleus (CN). In guinea pigs, a large percentage of the olivary cells that project to the CN are located in the medial somatotopic zone (MNTB). We have used fluorescent tracers (Fluoro-Gold, Fluoro-Ruby) to determine whether MNTB principal cells, which are post-synaptic to the large synaptic terminals known as calyces of Held, contribute to this projection. Injection of one of the CN labels cells in the ipsilateral MNTB and calyces of Held in the contralateral MNTB. By injecting different tracers into the CN on each side, we could examine each MNTB for retrograde transport from the ipsilateral side and anterograde transport from the contralateral side. In every case, some of the labelled cells were enveloped by a labelled calyx of Held, identifying them as principal cells. It is unclear whether the remaining labelled cells were non-principal cells or were principal cells whose afferent calyx was unlabelled.

Our study demonstrates a projection from one CN to the contralateral CN via the calyces of Held and MNTB principal cells.

Supported by NIH grants RO1 DC00135 and F32 DC00005.

435.16
SYNAPTIC CONNECTIONS MADE BY THE SUPERIOR OLIVARY COMPLEX (SOC) IN THE INFERIOR COCCULUS (IC). AN EM AUTORADIOGRAPHIC STUDY. Gertchen E. Beckius and Douglas J. Oliver*.

We made injections of H3-hexone and wheat germ-IRP into either the lateral (LSO) and medial superior olive (MSO) of the adult cat. The nuclei were located by stereotactic coordinates and by their response to binaural tones. EM autoradiographs from the IC were prepared with Bifred L4 emulsion and exposed 4-66 wk. Axonal endings from the contralateral LSO usually contain round synaptic vesicles and make asymmetric synapses (RA endings) in the IC. However, three third have pleomorphic synaptic vesicles and make symmetrical contacts (PS endings). Endings from the ipsilateral LSO are more numerous and varied. About half of this projection to the IC is RA endings: 45% are RA endings; 5% are unusual with pleomorphic vesicles and asymmetrical contacts (PA endings).

Overall, endings from the SOC represent the largest source of RA endings for the IC. The MSO and LSO projections make up 33% and 26% of all the presumed excitatory inputs to the ipsilateral IC, but the projection from the contralateral LSO represents only 17%. The bilateral LSO projections account for 38% of the presumed inhibitory PS endings.

These data support the concept that synaptic domains are distinct functional zones where different types of inputs are segregated in the IC. The sum of all RA endings from cochlear nucleus and superior olive exceeds 130% of the RA endings in IC. In addition, PS endings from dorsal nucleus of the lateral lemniscus and LSO total 76% of all PS endings in IC. The surplus of RA endings suggests that some excitatory inputs to the central nucleus are mutually exclusive and do not overlap on the same postsynaptic neurons.

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436.2

In order to examine the contributions of Na+, HCl and receptor mechanisms to the responsiveness of cells in the nucleus of the solitary tract (NST), we have determined concentration-response functions for a wide array of chemical stimulants. This array includes 8 sodium salts, 2 lithium salts, 5 sodium acids, 5 acids, 5 sweet-tasting stimuli, and 5 bitter-tasting stimuli. We recorded multunit activity from the NST evoked by anterior tongue stimulation with 5 or more concentrations of each tastant. The peak of the integrated multunit response to a 10 sec test stimulus was measured; these values were expressed as proportions of the peak response to a 0.02M NaCl standard. The slopes of the concentration-response functions derived in this way varied with respect to taste quality and chemical composition. In particular, the slopes for some salts were more steeper than for NaCl, some were less steep, yet many stimuli were similarly effective at 0.05M. The data for salts are well described by power functions and the slopes for the same functions for MNTB to human psychophysical studies on the anterior tongue. Equally effective concentrations are chosen from these functions for single unit studies; the matching concentrations (M) for stimuli determined to date are: NaCl=0.032, NaOAc=0.032, NaSO4=0.032, Na-acetate=0.032, L-glutamic acid=0.028, KCl=0.032, NaCl=0.012, citric acid=0.0026, tartaric acid=0.0018, Na-saccharin=0.0014, dextrose=0.73, sucrose=0.028, quinine-HCl=0.032 and urea=1.73.

Supported in part by NIDCD grant DC00353-07.
436.3

A PATCH-CLAMP ANALYSIS OF NEUROKINEIN RECEPTOR ACTIVATION IN THE GUSTATORY PORTION OF THE SOLITARY NUCLEUS.


In previous experiments, we have shown that substance P (SP) can excite many neurons in the gustatory portion of the nucleus of the solitary tract (NST) of the hamster. Using whole cell patch-clamp techniques in in vitro slice preparation, we have examined the effects of SP on the membrane properties of cells in the rostral hamster NST. Recordings were made from 21 cells with stable resting membrane potentials. Most of the cells fired repetitive action potentials with a mean spike overshoot of 64.7 ± 7.7 mV. Short application of the neurokinin receptor agonist depotilized 13 neurons with a mean depolarization of 4.5 mV that lasted for 1-2 min; in 10 cells this depolarization was accompanied by unitary conductance and an oscillating activity. Another 3 cells were hyperpolarized 4-6 mV with an increase in membrane conductance and a decrease in spontaneous firing. The remaining 5 cells were not affected by SP. In the presence of TTX, the depolarizing effect of SP was not abolished by perfusion of the slices with high Mg2+/low Ca2+ PSS, indicating a direct postsynaptic action of SP. The effect of SP was blocked by its antagonist (D-Pro2, D-Trp7) substance P in 6 cells. Current/voltage curves indicated a conductance decrease during the depolarization with a reversal potential of the SP-dependent current close to the K+ equilibrium potential. We conclude that activation of neurokinin receptors decreases K+ conductance, leading to membrane depolarization, which may interact with gustatory afferent processing. Supported in part by NIDCD Grants DC-00353 and DC-00066.

436.5


Rats with lesions centered in the gustatory zone of the parabrachial nucleus (PBN) are able to respond to sucrose in a concentration-dependent manner, but this is sometimes blunted. This study examined the extent to which PBN lesions alter taste-guided avoidance of an aversive taste stimulus, quinine. Water-deprived rats were tested in a specially-designed apparatus, for their licking responses to water and 7 concentrations of quinine hydrochloride (0.003 mM to 0.05 M), during separate training and test trials. Water rejection preceded each stimulus trial and testing occurred over three 90 min sessions. Next, six deeply anesthetized rats received electrophysiologically-guided bilateral electrolytic lesions of the gustatory zone of the PBN. Two lesions were placed on each side, one at the ventral border of the area (60 µA, 20 s), and one 200-300 µm more dorsal (40 µA, 20 s). A second group (n = 13) received similar lesions, but the current for the dorsal lesion was doubled. The rats were then tested for sucrose controls. After recovery rats were tested for quinine responsiveness. In all groups, licking to quinine significantly decreased as a function of concentration and after surgery. There was a slight rightward shift in the concentration-response curve after sucrose-dt 9 log units to the right and the higher current-induced lesions caused a more substantial shift of about 1 log unit. Histological analysis is in progress to determine the relationship between the degree of behavioral impairment and the cytoarchitectural lesion parameters. These results suggest that rats with PBN lesions are not completely averse to quinine. These lesions, however, markedly attenuate taste-guided avoidance responses. Supported by PHS grant DC-00161.

436.7


In an automated gustometer, 12 rats generated concentration-response functions for 8 rapid chemicals and sucrose. When water deprived, the rats progressively decreased their licking of citric acid, MgCl2, NH4Cl, quinine HCI, and capsaicin. When replete, the licking of sucrose and quinine increased and capsaicin decreased. When water deprived, rats decreased their intake of NaCl and monosodium glutamate. Six of these animals and 3 additional, inexperienced rats had electrophysiologically guided lesions centered on taste neurons in the nucleus of the solitary tract (NST); 4 others (3 exp., 1 inexp.) served as controls. Subsequently, all animals were retested using the same stimuli under the same condition. Controls maintained their taste responses, but the lesioned rats exhibited consistent deficits in responding to rapid stimuli. Licking of 1.0 M sucrose did not differ from water; however, the composite substance responses remained about half as much as before surgery. Intake of capsaicin was not influenced by the NST lesions. In a second experiment, 3 preparations of 0.3 M alanine were paired with injections of LiCl (1.5 mM/kg, 0.15 M). Both the lesioned and the control rats learned to avoid the alanine. Thus, rats with NST lesions show a marked change in gustatory preference and aversion, but still can use taste cues for learned aversions. With lesions in the parabrachial nucleus, the second central taste relay, rats exhibit only modest changes in gustatory preference, but cannot learn a taste aversion. Supported by DC-00240, DC-00047, MH-06553.

436.6

RAT TASTERS AND NON-TASTERS REVEALED IN NEURAL ACTIVITY. B.K. Giza*, T. B. Scott & L. Zhang. U. Delaware, Newark DE 19716

Preferences for the bitter sweet taste of NaSaccarin (Sac) in rats may be related to the distribution of PROP sensitivity, as it is in humans. Do differences in NTS taste-evoked activity mediate these preferences? We offered rats water and 0.02M Sac, those selected those, those rats selected Sac (S) or >75% water (W). We recorded the activity of 56 cells from S- and 43 from W-rats in response to 13 stimuli. Activity in the two groups differed for four but greater for all other stimuli in W-rats. Thus water-prefering animals were not insensitive to sweet stimuli, but rather hyperresponsive to water. The two cell groups divided cells of each group into three clusters: sugar-, sodium- and acid-oriented. Sugar and sodium cells showed few differences between S- and W-rats. Rather, hyperresponsiveness of W-rats was most evident among acid cells. Therefore, hyperresponsitivity to non-sweet tastes in W-rats was carried primarily by cells whose activity is associated with aversive chemicals. The activity profile evoked by Sac was similar to those of sugars in S-rats, but more like those of non-sweet stimuli in W-rats. In humans, the sweetness of Sac does not differ between non-tasters of PROP, but bitter sensitivity is greater in the former group. It is the high bitterness that leads to its rejection by PROP tasters. A corresponding situation would seem to obtain in rats. Supported by research grant DK39064 from the NIDDK.

436.8


Single neurons were isolated from the rostral portion of the solitary tract of awake, behaving rats (n=41), during (n=58), and after (n=12) they were placed on a sodium-free diet. Fluid stimuli (30 µl) were delivered to the intragastric and intracardial veins. During deprivation, taste responses generally were reduced. Spontaneous activity increased by an average of 42%, while responses to water dropped by 28%. Mean response to NaCl decreased 47%; to sucrose, 59%; to citric acid, 32%; and to quinine HCl, 16%. Nevertheless, the response profiles of the neurons to 4 standard stimuli were not changed by the dietary conditions. In the depletion condition, 61% of the activity elicited by NaCl occurred in Na-best cells, 33% in sucrose-best neurons. In the depleted state, the figures were 60% and 26%, respectively. At higher stimulus concentrations, however, the relative responsiveness was altered by sodium deprivation. When the animals were sodium replete, in sucrose-best neurons, 1.0 M NaCl elicited only 60% as much activity as their Na-best activity. When the rats were sodium deprived. Although the sample was small, these results appeared to reveal toward previous values when sodium was added back to the diet. Supported by PHS grants DC-00240, MH-43787, MH-06553.
436.9 CYCLIC SPONTANEOUS ACTIVITY IN TASTE-RESPONSIVE UNITS IN THE NUCLEUS OF THE SOLITARY TRACT IN THE RAT. Scott Monroe* and Patricia M. Di Lorenzo. Dept. of Psychology, P.O. Box 6000, SUNY at Binghamton, Binghamton, NY 13902-6000.

Recent studies have shown that some taste-responsive units in the periaqueductal pons (PNb), the second relay in the central taste pathway in the rodent, show rhythmic, cyclical variations in spontaneous firing rate. The present study examined spontaneous activity in taste-responsive units in the nucleus of the solitary tract (NTS), the first relay in the central taste pathway, in the urethane-anesthetized rat. Initially, responses to representatives of the four basic taste stimuli were recorded followed by 15 min of spontaneous activity. Spontaneous activity (5 sec bins) was analyzed for cyclical characteristics and a Fourier analysis. Linear trends were removed prior to spectral analysis. Spontaneous activity was classified as cyclic if a peak in the activity spectrum existed that exceeded a 3% criterion. In 24 taste-responsive units that met this criterion, oscillatory activity not related to ingestion. Preliminary results show that 4 of 12 taste-responsive units showed significant cyclic spontaneous activity. These units showed single peaks in the spectral analysis ranging from 3.34-6.43 cycles/min, with a median of 1.8 cycles/min. Ten of the NTS units were recorded simultaneously with taste-responsive PnB units and in five of these pairs one unit showed cyclometry where the other did not. This suggests that the occurrence of cyclicity of spontaneous rate may be independent in the NTS and PnB. In contrast to the PnB units, spontaneous firing rates in NTS units were not suppressed following taste stimulation.

Supported by a grant from the Whitewall Foundation to P.D.i Lorenzo.

436.10 THE EFFECTS OF A CTA ON GUSTATORY EVOKED ACTIVITY IN THE RAT NTS REMAIN AFTER BEHAVIORAL EXTINCTION. L.J. Nolan* and T.R. Scott, Dept. of Psychology, University of Delaware, Newark, DE 19716.

Chang and Scott (1984) reported modification of gustatory evoked activity in the rat NTS as a consequence of a CTA. We investigated whether this modification would be lost with complete behavioral extinction of the aversion. Rats were conditioned to avoid .0025M sodium saccharin (CS) by pairing its taste (15 daily sessions). Initially, responses of the four basic taste stimuli were recorded followed by 15 min of spontaneous activity. Spontaneous activity (5 sec bins) was analyzed for cyclical characteristics and a Fourier analysis. Linear trends were removed prior to spectral analysis. Spontaneous activity was classified as cyclic if a peak in the activity spectrum existed that exceeded a 3% criterion. In 24 taste-responsive units that met this criterion, oscillatory activity not related to ingestion. Preliminary results show that 4 of 12 taste-responsive units showed significant cyclic spontaneous activity. These units showed single peaks in the spectral analysis ranging from 3.34-6.43 cycles/min, with a median of 1.8 cycles/min. Ten of the NTS units were recorded simultaneously with taste-responsive PnB units and in five of these pairs one unit showed cyclometry where the other did not. This suggests that the occurrence of cyclicity of spontaneous rate may be independent in the NTS and PnB. In contrast to the PnB units, spontaneous firing rates in NTS units were not suppressed following taste stimulation.

Supported by a grant from the Whitewall Foundation to P.D. Di Lorenzo.


The posterior lingual taste buds located within the foliate (FOL) and circumvallate (CV) papillae account for the majority of tongue taste buds in the rat (46% and 35%, respectively). However, there is a paucity of data on central processing of taste-responses arising from the posterior tongue, especially the CV. In the present study, in rat, the location of neurons responsive to specific CV and FOL stimulation were mapped within the nucleus of the solitary tract (NST) using standard extracellular recording techniques. Both posterior taste responses extended from rostral to about the level where the NST meets the 4th ventricle, with a trend for CV responses to extend caudal to FOL responses. In most cases, CV and FOL taste responses were recorded in the same electrode track, with FOL responses located dorsal to CV responses. Tactile responses from these two fields were coextensive. The majority of posterior tongue responsive sites, but were more widespread. All multinunit sites and four single units responsive to specific CV stimulation had similar response profiles: 0.01M HCI > quaternary mixture > 0.03M quinine+HCl, with no response to 0.3M NaCl and 0.01M Na saccharin. This central response profile is more specific than glossopharyngeal nerve response profiles previously described, but is in agreement with the robust responsiveness of this nerve to acids and alcohols.

Supported by NIH grants DC00416 and DC00417.

436.12 DISTRIBUTION OF TASTE-RESPONSIVE NEURONS IN THE HAMSTER SOLITARY NUCLEUS. A.P. Knox*, L.D. Savoy, T.P. Hettinger and M.E. Frank, Department of Biocomputer and Function, Univ. of Connecticut Health Center, Farmington, CT 06030.

The distribution of taste-responsive neurons in the rostral solitary nucleus (SN) was studied in anesthetized hamsters (Mesocricetus auratus). The anterior tongue was isolated in a flow chamber and seven taste stimuli (0.03 M NaCl, 0.03 M saccharin, 0.1 M glycine, 0.1 M KCl, 0.03 M Na acetate, 0.03 M NaCl (0.1 M sucrose) applied. Extracellular recordings from SN neurons were made with glass microelectrodes (1.8 MΩ) filled with 4% HRP in 0.5 M KCl and 0.05 M Tris buffer. Units were recorded on a VGR and played back through a window discriminator for data analysis. Successful recording sites were marked by ionophoresis of HRP, which filled stellate and elongate cells. Postmortem histological analysis showed frozen sections in the transverse, parasagittal or horizontal plane. The SN is prominent and can be reconstructed from sections in any plane. Sections were processed for HRP, counterstained and the taste-responsive area containing small, densely packed cells was mapped. A high percentage of cells in this area responded to one or more stimuli. Rates as high as 100 spikes/s were obtained to sucrose. Responses in the rostral SN may be stimulus selective at any one multinunit site. Single units, however, could display disparate responses at contiguous sites. These data argue that taste responsiveness of neurons may have a "modular" organization that selects for basic taste stimuli. This work was supported by NIH grant DC 06063.

436.13 TRANSGENOMIC DEGENERATION IN THE GUSTATORY SYSTEM. L.T. McGlathery and M.O. Whitehead*. Department of Surgery, Division of Anatomy, UCSF, La Jolla, CA 92037.

Peripheral taste nerves are prone to damage in humans and have been experimentally altered in animal studies of the defferentated gustatory system. It is not known whether peripheral nerve damage in the mammalian taste system results in degeneration of central axonal endings as in other sensory systems (e.g. trigeminal, vestibular, auditory). We studied 11 Golden hamsters whose right chorda tympani were severed in the middle ear. In 8 cases a control, fornemcretol, is applied to the nerve stump. The animals survived for 2-15 days. The brains were dissected and stained for degenerating axons and endings using the Fink-Heimer method. In all cases there was evidence of degenerating ending in the central nucleus of the solitary tract. Degeneration was concentrated in the chorda tympani terminal field in animals treated with both the nerve stump and fornemcretol. The degeneration was dense, especially at 11-13 days. In cases where the chorda tympani had been severed, degeneration was less robust but relatively heavy at 11 days and observable as early as 0.3 M NaCl. The degeneration observed after fornemcretol treatment could result from the death of pepticeptase cells. However, degeneration after single nerve cut is best explained as transganglonic, i.e. necessarily requiring cell death. The present findings indicate that peripheral nerve damage can profoundly disrupt the synaptology of the NST. Altered synaptology could relate to the dysaesthesia or other taste system of central origin reported for some patients after trauma in the palate, oral or oral surgery. (Supported by NIH Grant DC00452).


Research on alcohol consumption has generally employed water-alcohol mixtures, but in the real world alcohol is often flavored with another pleasant taste, often something sweet. Alcohol-saccharin mixtures were used in this study to achieve this goal and to determine the neural basis of this attraction. Subjects were extinguished by pairing 4% alcohol and 2% saccharin mixtures with body weight gain or body weight loss, respectively. Partial extinction was achieved by pairing saccharin and alcohol mixtures with the CS. However, when alcohol and saccharin mixtures were paired, the alcohol was not consumed. Alcohol-saccharin mixtures were given in the presence of water. The rats were then allowed to lick for 30 min lick analysis tests; volume consumed was measured at the end of each session. Solutions of saccharin (0.06 M) and alcohol mixtures (3%, 6%, 9%, and 12%, v/v) were presented in ascending and descending orders, one solution day each. Contrary to previous findings with water-alcohol mixtures [Kiefer et al., Alcohol, 4, 1987] rats lacking gustatory cortex did not consume more alcohol-saccharin at any concentration compared to controls. Preliminary analysis of lick rate also revealed no significant differences between groups. It is suggested that the presence of alcohol contributes to the lack of differences. There were significant differences across concentrations, with the CS consumed more saccharin and 3% alcohol-saccharin than other concentrations.
437.1

Autoradiographic studies have identified high levels of excitatory amino acid (EAA) receptors in the striatum. However, the localization of EAA receptor subtypes to specific populations of striatal projection neurons has not been determined. We used quantitative autoradiography to examine the cellular localization of NMDA, AMPA, metabotropic and kainate EAA binding sites in the striatum following selective lesion of striatoniigral projection neurons. Denervation of striatoniigral neurons was induced unilaterally by injection of the suicide transport toxin, kainic acid (0.5-0.8 ng in PBS), into the left substantia nigra. Following a survival period of 12 days, we observed a reduction of all EAA binding site subtypes in the striatum ipsilateral to the injected nigra. Striatal NMDA binding sites were reduced approximately 50%. The other EAA binding site subtypes exhibited more modest reductions; up to 16% for AMPA, 27% for metabotropic and 15% for kainate.

These results indicate that there are NMDA, AMPA, metabotropic and kainate binding sites on striatoniigral projection neurons and suggest that the NMDA subtype may be selectively enriched on striatoniigral neurons. Supported by NS13000, NS19613, NS07222 and the Hereditary Disease Foundation.

437.3

Various neuron types may be at risk during the course of Huntington's Disease (HD). Glial cells have not been extensively studied during the course of neurodegeneration within the structure most involved in the disease process, the caudate-putamen (or neostriatum). Since the astrocyte could be a cell at risk, or even associated with the primary defect, we examined two markers that have been shown to be specific for astrocyte "reactivity," (galilgal fibrillar acidic protein, GFAP), and development, tenascin, a constituent of the extracellular matrix that is developmentally regulated in the neostriatum and other structures, and also expressed early in CNS injury).

Immunocytochemistry for GFAP and tenascin using well-characterized monoclonal and polyclonal antibodies, was carried out on presymptomatic, and Grades 2& 3 HD striatum (using the grading scale of Vonsattel). Our studies have revealed that astrocytes show changes in the presymptomatic HD tissue, and that their reactivity, distribution, and expression of tenascin and GFAP mirrors the degenerative process through the caudate and putamen as the disease progresses. In particular, we have found that GFAP and tenascin are upregulated in the HD striatum, and distributed in a patchy manner that may either represent neurons or a matrix compartment. In presymptomatic tissue, GFAP and tenascin patchy staining is light, as compared to Grades 2 & 3. By Grade 3, the tenascin and GFAP immunostaining in the caudate and putamen is dense and pervasive. In Grades 2 & 3, GFAP staining is associated with classical reactive astocytes, but in the presymptomatic tissue the GFAP labels what resemble immature astocytes. Thus, astocytes exhibit morphological and biochemical changes that may be associated with the onset of disease. Supported by the Hereditary Disease Foundation.

437.4

Previous in vitro electrophysiological studies have shown that DA increased the spontaneous firing of GABA interneurons in the medial frontal cortex (MFC). This activation could be involved in the inhibition of the firing rate of pyramidal cells observed in vivo following DA neuron stimulation of the ventral tegmental area (VTA). The DA control of MFC GABA interneurons was further investigated by studying the spontaneous 3H-GABA release on MFC slices. Three D2 agonists (quinpirole, RU 24969 and butrodone) dose dependently enhanced 3H-GABA release. These effects were antagonized by the classical D2 antagonist sulpiride. Endogenous DA release by amphetamine also increased the release of 3H-GABA through a D2 receptor activation. A permissive effect of D1 agonist was observed on the D2-mediated increase of 3H-GABA release. The D2 activatory effect observed on GABA interneurons of the MFC was antagonized by the inhibitory effect observed on the spontaneous 3H-GABA release in the striatum.

Finally, DA regulated the levels of mRNA encoding one of the isoforms of glutamic acid decarboxylase (GAD67, GAD65) which showed that the electrotic lesion of VTA neurons decreased GAD67-mRNA expression in the MFC. At the opposite, a 6-OHDA lesion of DA nigral neurons enhanced GAD67-mRNA levels in the striatum, an effect which can be reproduced by a chronic treatment with sulpiride and haloperidol.

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437.5 SCOPOLAMINE ATTENUATES HALOPERIDOL-INDUCED C-FOSEXPRESSION IN THE BRAIN. N. Guo,1 S. Robertson and H.C. Fibiger, Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, 2255 Westbrook Mall, Vancouver, B.C. Canada, V6T 1Z3. Haloperidol-induced lesions in some parts of the central nervous system. Haloperidol also induces catechol in rodents and extrapyramidal side effects in humans, both of which are reduced by muscarinic antagonists. In order to gain insight into the neurochemical and neuroanatomical substrates of haloperidol-induced catechol we examined the effects of the muscarinic receptor antagonist scopolamine on haloperidol-induced C-fose expression in the striatum, nucleus accumbens, and lateral septal nucleus. Male Wistar rats received subcutaneous injections of scopolamine (2.5mg/kg), or vehicle (1mg/kg) 30 minutes before haloperidol (2mg/kg). The rats were perfused 2 hours after the injection. Immunohistochemical staining was performed on brain sections (30μm). We found that the scopolamine-induced C-fose expression was significantly reduced to half of the haloperidol-induced levels.

437.6 METABOLIC EFFECTS OF CONTINUOUS L-DOPA INFUSION IN RATS WITH UNILATERAL SUBSTANTI NIGRA LESIONS. G.A. Hubbard, J.E. Bennett and J.M. Trugen, Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908. We used the 2-deoxyglucose (2-DG) method of measuring regional cerebral glucose utilization (RCGU) to study the effects of continuous L-dopa infusion in rats with unilateral 6-hydroxydopamine nigral lesions. Rats were implanted with osmotic minipumps (Alzet, ALZ-2001, 1000 μl/h, 24 h) with L-dopa (250, 500, or 1200 mg/kg/day) or saline. L-dopa and 2-DG uptake in the subthalamic nucleus (up 40%) and significantly increased RCGU in the dorsolateral striatum (up to 50%) in the lesions were observed. L-Dopa infusion was shown to be beneficial in the treatment of Parkinson's disease.

437.7 PROGRESSION OF CEREBRAL METABOLIC CHANGES IN MPTP-INDUCED HEMIPARKINSONISM IN MONKEYS. E. Polombo,1 K. Brandt,2 J. Segal3 and J. Portier1.1 MNI, Bethesda, MD 20892; 2NIH, Bethesda, MD 20092; 3Howard University School of Medicine, Washington, DC 20060. Local rates of glucose utilization (LCGU) were determined in hemiparkinsonism monkeys, 6-16 weeks (short-term) or 16 to 26 weeks (long-term, n=3) after unilateral intracarotid infusion of MPTP. Four additional monkeys served as normal controls. MPTP-treatment resulted in similar clinical signs of hemiparkinsonism, e.g. unilateral bradykinesia and rigidity that were apparent in all 6 treated monkeys. In the short-term group, significant side-to-side asymmetries were restricted to the substantia nigra compacta (SNC) medialis and to the zona incerta (ZI). Additional smaller differences were seen in the external globus pallidus (GP) and subthalamic nucleus (STN). In the long-term group, LCGU decreases in the SNC medialis were similar (31% short-term vs 34% long-term). Changes in the GP (+30 vs +55%), STN (14 vs 20%) and ZI (+9 vs +18%) were far more pronounced. In addition, LCGU was increased in the dorsolateral caudate (+18%), all of the putamen (+25%), and pedunculopontine nucleus (+19%) ipsilateral to the lesion. Furthermore, bilateral reductions were present in the ventral tegmental segmental area as compared to controls. Bilateral increases were also present in the pars oralis of ventral striatum and in the supplementary motor area. Within the time frame of this study, the pattern of LCGU changes in the basal ganglia and associated structures becomes more complex as the length of time after Snpc denervation increases. This suggests that pathophysiological events in the brain progress in spite of a relatively stable clinical syndrome.

437.9 EFFECTS OF CHLOROZAPATINE TREATMENT ON INDUCTION OF FOCALLIKE PROTEINS IN THE STRIATUM. N. Haan1 and A.M. Graybiel. Department of brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139. We have previously reported that acute injection of the atypical antipsychotic drug clozapine (CLZ) induced Fos-like immunoreactivity (FLI) in neurons of the nucleus accumbens and caudoputamen acutely (by 2 hrs) in a disease-dependent manner (Ferri et al. Soc. Neurol. Abstr., 1991). We have now compared the effects of acute CLZ treatment (>8 days) and clozapine treatments. Groups of rats received one of the following treatments: chronic CLZ followed by acetic acid (AA), or chronic CLZ followed by vehicle (150 mg/kg, i.p.), and on the 9th day they received acute injection of CLZ (20 mg/kg, i.p.); or vehicle (10 mg/kg, i.p.) and vehicle (900 mg/kg, i.p.) followed by AA (150 mg/kg, i.p.). The rats were perfused 2 hours after the injection. The results showed that chronic treatment with CLZ changes the gene-expressive responses of nucleus accumbens neurons such as acute nuclei and 2 dose changes may reflect regulation of Fos-like antigen expression by the monocytes to visualize Fos protein. FLIs that decreased the expression of the neuronal Fos antigen, supported by the Human Frontier Science Program and Javits Award NIA. We thank Dr. F. Sharp for a Fos monoclonal antibody and Sandoz Pharmaceutical for a gift of drug.

437.10 LOCAL INJECTION OF DOPAMINERGIC AND MUSCARINIC AGONISTS MODULATE STRIATAL PEPTIDE GENE EXPRESSION. L.K. Nielsen,1 S.T. Krau, and C.R. Geffers. Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis, Memphis, TN, and Lab of Cell Biology, NIMH, Bethesda, MD. The antagonistic balance between acetylcholine (ACh) and dopamine (DA) within the striatum has long been recognized to be important in the functional activity of the basal ganglia. In order to investigate how ACh and DA influence the output of dopaminergic (DA) and mesolimbic projection neurons, we have examined the regulation of neuropeptide gene expression by dopaminergic and muscarinic agonists. Following the activation of the nigrostriatal dopamine pathway, there is an increase in the expression of enkephalin mRNA in striatal projection neurons, while the level of dopamine mRNA remains unchanged. The production of locomotor activity by the application of D-1 and D-2 agonists further modifies the expression of dopamine and enkephalin mRNA in the striatum of lesioned rats. In order to test whether the action of these drugs is mediated locally within the striatum, SKF-38393, a D-1 agonist, was injected directly into the striatum or lateral ventricle (cve) of 6-OHDA lesioned rats. In situ hybridization histochemistry was used to measure the expression of dopamine and enkephalin mRNA. Injection of 5 μg SKF-38393 directly into the striatum produced a local increase in dopamine mRNA surrounding the injection site. Likewise, 1-10 μg SKF-38393 (100% increase) and 5 μg SKF-38393 (100% increase) increased enkephalin mRNA within the dorsal medial striatum adjacent to the ventricle. Next, the effect of a muscarinic agonist on peptide expression in 6-OHDA lesioned rats was examined. Injections of pilocarpine (120 μg) icv resulted in a further 25% increase in enkephalin mRNA levels, while a lesion-induced level in striatal neurons within the dorsal medial region adjacent to the ventricle. We conclude that the effects of both D-1 and muscarinic agonists on peptide expression are mediated locally within the striatum. Further, this study shows the importance of DA and ACh receptor interaction in the striatal gene expression of striatal neurons.

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437.11
TIME COURSE OF ALTERATIONS IN SUBSTANCE P, DYNORPHIN, ENKEPHALIN AND o-FOs GENE EXPRESSION IN STRIATAL NEURONS DURING CHRONIC COCAINE TREATMENT.
H. Steinert* and C. R. Garden, Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.
Projection neurons of the striatum differ in the expression of neuropeptides: striatonigral neurons mainly express substance P and dynorphin, whereas striatopallidal neurons express enkephalins. Dopamine modulates the levels of these peptides. We examined with in situ hybridization histochemistry short-term and long-term changes in glibergic expression of these peptides, as well as of the immediate-early gene c-fos, during chronic treatment with the indirect dopamine agonist 7-OH-DCPP (1 mg/kg, i.p., twice daily). Thirty minutes after acute application of cocaine, mRNA levels of substance P and c-fos are significantly increased, mostly in dorsal striatal regions. On day 3 of the chronic treatment, the cocainia-induced increase in mRNA levels is significantly reduced (as compared to the levels at the beginning of the treatment), and remains reduced thereafter, in most regions. Conversely, mRNA levels of dynorphin and enkephalin are unchanged 30 min after the acute cocaine application, but are significantly increased on treatment day 2, also predominantly in dorsal regions, and remain elevated in some regions after a longer treatment. These results show that chronic cocaine treatment produces different temporal and regional patterns of alterations in gene expression in striatonigral and striatopallidal neurons.

437.12
Suicide transport agents elicit regenerative degeneration of neurons projecting to an injection site. In the basal ganglia these toxins have been shown to produce selective lesions of the striatopallidal and striatonigral pathways based on receptor binding and neurochemical mRNA studies. In the present immunohistochemical study, the neostriatal of adult rats were examined 10 days after an injection of volkine or into the substantia nigra (SN) or an injection of OX-7 aminophylline into the globus pallidus (GP) (n=5). Adjacent sections were processed for (1) Nissl stain to study the density of all neurons and large interneurons, (2) NADPH-diaphorase (4) histochemistry, to mark medium-sized interneurons, (3) enkephalin (ENK) immunocytochemistry (ICC), to label striatopallidal neurons, or 4) substance P (SUB) ICC to label striatonigral neurons. Analyses of Nissl stained sections revealed that the striatal ipsilateral to the lesions appeared healthy and did not exhibit shrinkage or gliosis; however, a moderate decrease in cell density was detected by quantitation (33-44%). The densities of large neurons and NADPH-di-labeled neurons in the ipsilateral striata were unchanged after OP lesions and showed a significant decrease (70%) after FN lesions. After OP lesions the density of ENK-labeled cells (21%) decreased more than that of SUB-labeled cells (16%). Conversely, after FN lesions, the density of SUB-labeled cells (56%) decreased and more than that of ENK-labeled cells (20%). These data suggest that interneurons are relatively spared and that projection neurons may be differentially affected after OP and FN lesions. Supported by DRIF, U. of MD (RCR) & NS01544 (MBH).

437.13
IMMUNOHISTOCHEMICAL LOCALIZATION OF 3-HYDROXYANTHRANILIC ACID OXGENASE (3HAAO) AND KYNURENE AMINOTRANSFERASE IN ASTROCYTES IN THE RAT SUBSTANIA NIGRA. R. Schwartz*, F. Da, K.E. McCarthy, E. Okano and R.C. Roberts. Maryland Psychiatric Research Center, Baltimore, MD 21228.
Endogenous excitotoxins, such as quinolinic acid (QUIN), may be involved in the pathogenesis of several brain disorders. Kyneric acid (KYNA), an endogenous antagonist of excitatory amino acid receptors, has neuroprotective and anticonvulsant properties. The immunohistochemical localization of 3-hydroxyanthranilic acid oxygenase (3HAAO) and kyneric aminotransferase (KAT), the biosynthetic enzymes of QUIN and KYNA, respectively, was examined in the adult rat substantia nigra, pars compacta (SNpc). At the light microscopic level, KAT and KYNA immunoreactivity (ii) were present in glia in a robust and homogenous pattern in both the nucleus and cytoplasm. At the ultrastructural level, both 3HAAO and KAT i were present in astrocytic processes surrounding capillaries. 3HAAO was abundant throughout the neuron in fine calibre glial processes which often encased synaptic profiles, both asymmetrical (erytactic) and symmetrical (inhibitory). KAT i was also present in astrocytic processes, but usually of larger calibre than those labeled by 3HAAO antibodies. KAT-i-glial processes abutted, rather than surrounded, both symmetrical and asymmetric synapses. Thus, astrocytic QUIN and KYNA appear to be in a position to modulate excitatory amino acid receptor function in the SNpc. Supported by USPHS grants NS28236 and MH4211 and a HSDA fellowship (P.D.).

437.14
Acute nicotine injections induce c-fos expression mostly in nondopaminergic neurons of the ventral tegmental area (VTA). Ying Pang, Hideo Kiba, H. Gould* and A. Jayaraman, Dept. of Neurology, LSU Sch. of Med., New Orleans, LA 70112.
Induction of c-fos gene is considered to be an immediate and early response in the cascade of molecular events that ultimately lead to long-term alterations in gene expression in neurons. The psychomotor stimuli and positive reinforcing effects of nicotine have been speculated to be mediated by the dopaminergic neurons of the VTA. To identify the precise subpopulation of the dopaminergic neurons, the pattern of expression of c-fos gene was mapped using immunohistochemical methods.
Acute nicotine injections (0.3-1.4 mg/kg, i.c.) resulted in a prominent Fos-like immunoreactivity (Fos-li) in the interpeduncular nucleus, medial terminal nucleus of the accessory optic tract and also prominently in the caudal linear subnucleus (SND) of the VTA. The neurons of VTA subnuclei, viz., the rostral linear, parabrachialis, nucleus parabrachialis pigmentosus, and nucleus interfacialis did not contain any cells. Fos-li. Mecamylamine abolished Fos-li in neurons of VTA, superficial layers of superior colliculus, periaqueductal gray areas and the interpeduncular nucleus.
These results suggest that acute nicotine injections induce c-fos expression mostly in nondopaminergic neurons of the ventral tegmental area. Supported by Smokeless Tobacco Research Council.

437.15
We have previously hypothesized that these striatal neurons expressing both high levels of glutamic acid decarboxylase (GAD67) mRNA are interneurons (Cheeslet and Robbins, 1989). To further test this hypothesis, striatal sections were hybridized simultaneously with a digoxigenin-labeled RNA probe for GAD67 and a 32P-RNA probe for parvalbumin, a calcium binding protein present in striatal GABA-ergic interneurons (Cowen et al. '90; Kita et al. '90). The majority of neurons densely labelled for GAD mRNA also expressed parvalbumin mRNA, confirming that they are interneurons. Previous studies have shown that dopamine depletion selectively decreased GAD67 mRNA levels in the densely labelled cells (Gougham et al. Brain Res. 1992). Further double-labelling experiments revealed the presence of mRNA for dopamine D2, but not D1 receptors in these neurons, suggesting that the D2 receptor subtype may be involved in the effects of dopamine on striatal GABA-ergic interneurons. Supported by PHS grant MH-44889. We thank A.J. Tobin (UCLA) and M. Berchtold (Zurich) for the cDNAs.

437.16
The processes underlying dopaminergic neurotoxicity produced by chronic administration of methamphetamine [m-AMPH] are incompletely understood. Insights into m-AMPH's cytotoxic mechanisms may be gained by examining the effects of a neurotoxic regimen of m-AMPH has on subpopulations of dopaminergic striatal projections. Using measures of dopamine content and [H]mazindol-labeled dopamine transport sites (visualized with quantitative autoradiography), we report differential vulnerability of striatal regions of rats given m-AMPH one week earlier (4 injections (s.c.), each 4 mg/kg, 2 hrs apart). Three rostrocaudal levels of the striatum were sampled (anterior, middle and posterior), and several regions were examined at each level (c.g. dorsal, lateral, ventral and medial regions; nucleus accumbens at the anterior level). Treatment with m-AMPH resulted in a significant decrease in [H]mazindol binding at both the anterior and middle levels, with the ventral and medial subregions exhibiting greater reduction than the dorsal striatum or the nucleus accumbens. Parallel to the heterogeneous reduction of striatal [H]mazindol binding by m-AMPH, striatal dopamine content depletion at the anterior level was most severe in the ventral region. The results encourage attempts to identify factors within the ventral-medial caudate nucleus that make it more susceptible to m-AMPH's neurotoxic effects as compared to the adjacent nucleus accumbens and the dorsal caudate nucleus.

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438.1 PERCEPTION OF DURATIONS OF KINESISTHETIC STIMULI IN CEREBELLAR PATIENTS. J.S. Lee, S.E. Grill, M. Balint, * Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892.

Coordinated movement depends upon proper temporal control of muscle activation. Persons with cerebellar disorders have difficulty with such movements as well as disturbances of durations of auditory and visual stimuli. We have compared the ability to perceive durations of kinesistic stimuli of seven patients with cerebellar disorders (without clinical evidence for peripheral neuropathy) to that of six normal controls. Subjects were seated with their right forearm resting on a table midway between the midline and slightly to the right of the body. The right index finger was fixed and the index finger placed squarely in a finger holder attached to a computer-controlled torque motor. This allowed imposition of controlled changes in joint angle about the metacarpophalangeal joint. After the completion of the presentation of a pair of joint angle steps two seconds apart, of different durations but the same magnitude (about one degree). Each pair of stimuli consisted of a standard 150 ms duration step and a variable duration step ranging from 50 ms to 250 ms. The standard was 150 ms long, and between 150 and 350 ms when the standard was 250 ms long. Stimuli were presented in random order. Subjects were instructed to report which stimulus was longer, by responding with "one" or "two". The session continued until each pair of stimuli was presented 10 times.

There were striking differences in the ability to distinguish the durations of stimuli between normal subjects and cerebellar patients (p<.001). For example, normal subjects were able to distinguish a 150 ms stimulus from one of 110 ms duration with only a 17% error rate, whereas cerebellar patients performed (making errors 46% of the time).

Impaired resolution of durations of kinesistic stimuli by motor centers may contribute to deficits in coordinated movements in persons with cerebellar disorders.

438.2 PERFORMANCE OF RATS ON A PERCEPTUAL TIMING TASK FOLLOWING CEREBELLAR LESIONS. J.V. Haido and R. Ivry, * University of California, Berkeley, CA 94720.

In previous research, we have found that patients with cerebellar lesions are impaired on motor and perceptual tasks that require precise timing. The present study used an animal model to investigate cerebellum in perceptual timing. Rats were trained on a two-choice discrimination task in which the duration of a visual stimulus was either 300 or 700 ms. Probe stimuli of intermediate durations were also presented without reward. Animals received either sham lesions or bilateral electrolytic lesions targeted at the lateral cerebellar nuclei. Preliminary results indicate: 1) the lesions have minimal effect on overall response rate; 2) the experimental and control animals perform similarly on the endpoint stimuli; and 3) the experimental animals are less consistent in their judgments of probe stimuli (differential discrimination). Further experiments and analyses with this paradigm should help clarify the role of the cerebellum in time perception.


In normal rats there is a decline in sensitivity to serotoninergic (5-HT) agonists during the second postnatal week. During this period, the genetically dystonic (d) rat, an animal model of inherited movement disorder, exhibits enhanced behavioral sensitivity to both quipazine and 8-hydroxy-2-(di-n-propylamino)tetrahydrobenzazepine (8-OH-DPAT) (Michela et al., 1990; Weland and Lucki, 1991). Since this is also the period during which the motor syndrome of the d rat appears, the enhanced sensitivity may be a secondary consequence of the movement disorder per se. To test this hypothesis, the behavioral effects of 5-HT agonists were examined in intact and cerebellectomized d and normal rats at 15-20 days of age. Earlier studies indicated that the output of the cerebellum is abnormal in d rats, and it has recently been found that cerebellectomy (CEREBELLA) eliminates the expression of the motor syndrome. Despite the improvement in motor ability observed after CEREBELLA in the d rats, 8-OH-DPAT produced a characteristic dose-dependent behavioral syndrome in all groups, and the enhanced sensitivity of the d rats was maintained when compared to normal littermates. Thus, the behavioral sensitivity of the d rats is not secondary to their movement disorder. Since experimentally-induced damage to the olivo-cerebellar system in normal rats also increases sensitivity to 5-HT agonists (Wieland, et al., 1990), the cerebellum may normally exert an inhibitory effect on the 5-HT syndrome that is absent in the d rats. (Supported by the Dystonia Medical Research Foundation.)

438.4 EFFECT OF CEREBELLECTOMY ON THE MOTOR SYNDROME OF THE GENETICALLY DYSTONIC RATS. M.S. LeDoux, J.F. Lorden, and J.M. Erwin, Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The dystonic (d) rat is an autosomal recessive mutant with a motor syndrome that resembles generalized dystonia seen in humans. In the absence of intervention, motor function deteriorates, resulting in death by postnatal day 35. Glucose utilization studies and electrophysiological recordings have demonstrated abnormal neuronal activity in the deep cerebellar nuclei of the d rat. To test the hypothesis that abnormal cerebellar output is responsible for the d motor syndrome, total cerebellectomies, including ablation of the cerebellar nuclei, were performed on d and normal rats at 15 and 20 days of age. As a control for non-specific lesion effects, a separate group of 15-day-old d rats received bilateral kainic acid lesions of the entopeduncular nucleus. Rats underwent behavioral testing just prior to surgery and again three days later. Separate groups of aged-matched normal and d rats served as unoperated controls. Behavioral testing included the determination of righting reflex times, locomotor and climbing ability. In addition, the severity of the motor syndrome was evaluated.

The d rat demonstrated an immediate and permanent improvement in motor function and a marked decrease in abnormal motor signs after cerebellectomy. Cerebellectomy allows the d rat to survive into adulthood and mate successfully. Interruption of basal ganglia output failed to improve the condition of the d rats. These findings suggest that cerebellar output is critical to the expression of motor signs in the rat disease. (Supported by NS5-01087 and the Dystonia Med. Res. Fdn.)

438.5 THE EYE OPENING TIME AS A FACTOR ACCELERATING MATURATION OF CEREBELLUM. KURINJE CELLS IN THE KITTENS. G. E. Passino, J. M. S. Becken, Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, 194223, St. Petersburg, Russia.

In experiments performed on two age groups of anesthetized kittens (i) blind kittens (3-5 days after birth) after eye opening (15-17 days) the activity of identified cerebellar Purkinje cells (PC) was studied. In blind kittens the cerebellar PC discharges represented by low frequency complex (CS) and simple spikes (SS) namely 0.37 - 0.48 and 3.5 - 3.7 imp/s respectively were recorded. A week after the eye opening (15 - 17 days) the frequency of CS and SS in PC discharge was increased approximately on three fold reaching 0.76 - 0.87 and 8.77 - 11 imp/s respectively, i.e. the velocity of the increase of PC discharge frequency before or after eye opening is expressed as 0.3. In blind kittens the cerebellar PC in response to stimulation of n.ishedidioc was no rule silent discharge whereas PC of (ii) group kittens responded even on the threshold stimuli by SS discharge followed by inhibitory complex (IC). In this case the duration of IP was longer compared with that seen in background active Purkinje cells.
438.7
TOPOGRAPHY OF SACCadic EYE MOVEMENTS IN THE CEREBELLMAR VERMIS OF THE RABBIT EVOKED BY MICROSTIMULATION.
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In the oculomotor nuclei of the cerebellum, the complex spiky movements of the cerebellar lobules VI and VII, saccades can be evoked with low intensity microstimulation (Noda and Fujikado, J. Neurophysiol, 1987). The direction of saccades is ipsilateral, with an upward component in the more rostral part of the oculomotor nucleus and downwardly when going caudally. In rabbits, eye movements have been evoked with microstimulation in other parts of the cerebellum, but not the vermis.

Rabbits were prepared, under full anesthesia, for recording microstimulation-evoked eye movements. Scleral search coils were implanted under the conjunctiva in both eyes and a recording chamber was placed over the cerebellum vermis. After full recovery, penetrations were made in the vermis, searching for eye movements with a 200ms train of biphasic 2ms pulses at 330Hz and a maximum current of 60μA. When eye movements were observed, the current was lowered to assess the threshold. At the end of the experimental period, localization of the stimulation sites was verified by histological analysis.

In parts of lobules VI and VII, sacadic eye movements were evoked by currents ranging between 4 and 60μA. The movements were horizontal with no apparent vertical component. In a strip of approximately 4mm width on either side of the vermal midline, the cortex could be divided in two equal longitudinal zones. In the medial zone sacades were directed ipsilaterally, in the lateral zone contralaterally. Thus the topography of sacadic eye movements in the cerebellum in rabbits is unlike that of monkeys.

438.8
Neuronal activity in lateral cerebellar vermis (lobulus simplex) of cats, related to visual stimuli at rest and during locomotion. D.E. Marple-Horvat, J.M. Craig and L.E. Armstrong. SPON: Brain Research Association, Department of Physiology, School of Medical Sciences, University, Bristol BS8 1TD

One of the major pathways from visual areas of the cerebral cortex to motor cortex travels via the pontine nuclei to the cerebellar cortex. This projection suggests that a major component of the visuomotor system is the vestibulo-ocular and other proprioceptive signals from labyrinths. In this study, we have observed that the cerebellum sets the frequency of burst discharges of Purkinje cells in cats that is related to visual activity.

438.9
OPTICAL IMAGING OF RESPONSES TO ELECTRICAL AND NATURAL FACE STIMULATION IN THE RAT CEREBELLUM.
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Afferent responses to the cerebellar cortex have been characterized as a "fascicular mosaic". In this study optical recordings with a voltage sensitive dye were used to evaluate the spatial patterns of activity evoked by afferent input. In anesthetized rats (ketamine/xylazine), the exposed cerebellar cortex was stained with RH790 for 2 hours. Using epifluorescence optics, Crs I and II were imaged with a Photometrics CCD system (14 bit A/D, 516 x 384 pixels). A saline filled chamber was used to control brain movement. Stimulation of the face consisted of either discrete biphasic electrical stimulation or "natural" stimulation by a Ling vibrator controlled probe. The basic experimental paradigm consisted of subtracting "background" images without stimulation from a corresponding "stimulus" image during which the face input was delivered. For both types of inputs optical signals could be obtained, characterized by patches of activity. Although small in size (0.1% of firing rate) the response was temporally consistent and spatially corresponding with the optical signals. Consistent with a granule cell layer origin the optical signals were recorded at depths of 400-500μm.

These results show that optical signals in the cerebellar cortex can be obtained with not only electrical but more physiological afferent inputs. (Supported by NIH grants NS-27210 and NS-18388).

438.11
INFLUENCE OF ARTERIAL PRESSURE ON PURKINJEE CELL ACTIVITY. L. Robertson*, Dept. Biological Structure and Function, School of Dentistry, Oregon Health Sciences University, Portland, OR 97201.

We have shown that a significant proportion of Purkinje cells in lobule V have complex spikes elicited by stimulation of the vagal and renal afferent nerves. This study tested the hypothesis that changes in the number and complexity of Purkinje cell spikes are modulated by changes in arterial pressure and by input from baroreceptors. We measured changes in spontaneous and spontaneously-elicited simple and complex spike activity during pharmacologically-induced increases in arterial pressure and during electrical stimulation of the buffer nerves in the decerebrate cat preparation (i.e., in absence of a hypothalamic-pituitary response to blood pressure changes). In about a third of the Purkinje cells tested, a nitroprusside-induced decrease in blood pressure resulted in a 75 to 90% reduction in simple spike activity. An arterial pressure decrease of 30 mercury or more was required for the reduction in simple spike activity to occur. The reduction in activity involved both peripheral elicited and spontaneous simple spikes, but did not involve the complex spikes. The reduced simple spike activity often persisted for several minutes after the arterial pressure had returned to baseline. The prolonged reduction of simple spike activity may reflect a long-term response by the visceral receptors to the decrease in arterial pressure, induced by the administration of phenylephrine, affected the spontaneous activity in only a few cells, some of which had a 25% increase in simple spikes and some had a 40% reduction. The relationship between decreased blood pressure and reduced simple spike activity supports a cerebellar role in cardiovascular regulation.

438.12
IN VIVO INTRACELLULAR RECORDING OF PURKINJEE CELL RESPONSES TO UPPER LIP STIMULATION IN CRUS IIA OF THE RAT CEREBELLUM. D. Jeng-Jen, L.M. Brown. Div. of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

Earlier work in our group has shown that a brief tactile stimulus delivered to the lip of a rat can lead to prolongation of burst discharges and complex spikes of Purkinje cells are modulated by changes in arterial pressure and by input from baroreceptors. We measured changes in spontaneous and spontaneously-elicited simple and complex spike activity during pharmacologically-induced increases in arterial pressure and during electrical stimulation of the buffer nerves in the decerebrate cat preparation (i.e., in absence of a hypothalamic-pituitary response to blood pressure changes). In about a third of the Purkinje cells tested, a nitroprusside-induced decrease in blood pressure resulted in a 75 to 90% reduction in simple spike activity. An arterial pressure decrease of 30 mercury or more was required for the reduction in simple spike activity to occur. The reduction in activity involved both peripheral elicited and spontaneous simple spikes, but did not involve the complex spikes. The reduced simple spike activity often persisted for several minutes after the arterial pressure had returned to baseline. The prolonged reduction of simple spike activity may reflect a long-term response by the visceral receptors to the decrease in arterial pressure, induced by the administration of phenylephrine, affected the spontaneous activity in only a few cells, some of which had a 25% increase in simple spikes and some had a 40% reduction. The relationship between decreased blood pressure and reduced simple spike activity supports a cerebellar role in cardiovascular regulation.

Climbing fiber responses (CFR) were recorded in the lateral hemisphere of the cerebellum of a monkey trained to perform both flexion and extension of the elbow in response to conditioned stimuli randomly presented. Differences in the CFR frequency were found in relation to the initiation of the movement depending on whether the monkey moves in response to the presentation of the external stimuli (triggered movement) or performs a self-initiated movement. With triggered movements CFR increase about 100 ms before the onset of movement. With self-initiated movements, this response is very small or even absent in this context. In another experiment, the monkey is rewarded only when movements are initiated during a second cue presented at a fixed delay after the go signal. In this 'time constraint' condition, CFR increase significantly when the velocity exceed 220 degrees/second and when the corresponding reaction time (RT) is between 250 and 275 ms. These relationships are not shown when the monkey does not have to use a precise timing. The link between CFR, velocity and RT is stronger in time constraint task. We would like to suggest that CF are activated in relation to their own oscillatory rhythm. In a time constraint task, repetitive external stimuli may induce a change in the spontaneous rhythm of the CF and allow synchronization of CFR especially when the cue is delivered in phase with the imposed rhythm. These factors could have much less influence on self-initiated movement. Finally when a precise timing is required by the delivery of a second cue, CF are best activated and related to the kinematic parameters of the movement. These results suggest that olivocerebellar input induces synchronized activity in the cerebellum allowing a more efficient control of movement initiation. Supported by MRC grant of Canada.

438.14 RESPONSES OF PURKINJE CELLS TO HINDLIMB JOINT ROTATION IN THE CAT. C. Gray, V. Percivalle* and R. Popple. Dept of Physiology, Univ. of Minnesota, Minneapolis, Minnesota, MN 55455.

To determine the relationship between the response properties of cerebellar cortical neurons and their locations in the cerebellum, we recorded from Purkinje cells in the anterior and posterior lobes during small amplitude passive rotations of the hind foot in barbiturate anesthetized cats. The 109 responsive cells were located in lobules I through IX between 1.4 and 4.2 mm lateral from the midline. 87% of the responsive cells were located in anterior lobules II and III and posterior lobes VII and VIII. 67% of the cells in the anterior III lobes in the posterior responded to both flexion and extension stimuli, the remaining responded to only one direction of rotation. Response to a movement depended on a cell's location. Cells in the anterior row of 2 lobes (3-4.2 mm) were excited at short latency (13-18 ms) by rotation in either direction. Medial cells generally exhibited longer latencies (25-28 ms) and longer response durations. In the posterior lobe where they tended to respond specifically to flexion and extension while in the anterior lobe those which were excited by movement in one direction were usually inhibited by the other. The results suggest the possibility of a functional mapping of movement within the spinocerebellum. Supported by NIH Grant NS21143 and the Human Frontier Science Program.


A number of models for velocity storage and integration of eye velocity signals have been proposed which use positive feedback to integrate signals related to changes in network or single unit parameters and a restricted number of units or a lack of variability related to the physiology of the vestibulo-ocular reflex (VOR). The aim of the present work was to study the properties of a model of bilateral neural network of the medulla oblongata which included several different types of units representing brainstem and cerebellar structures and two representing the horizontal ocular motoneurons. There were four inputs (representing two types of bilateral afferents) from the two horizontal semicircular canals. Each unit had a sigmoid input-output function and predicted the response to other head movements. The network was robust to perturbations in the connection weights and removal of a unit and predicted the response to other head movements. (Supported by NIH (DC00110) and Minn. Supercomputer Institute.)


Vertical vestibulo-ocular reflex (VOR) in cats tested in the 90° rolled position (vertical axis pitch) is less accurately compensatory than in the usual horizontal axis pitch). This inaccuracy could be related to a predominance of upward direction slow phase eye movements during low frequency rotations. Last year we reported a steady upward drift of the eyes in darkness in the absence of any rotation when the cat was in the 90° rolled position. Representing the drift as a velocity offset term allowed satisfactory sinusoidal fits to eye velocity during 2°/sec 0.01 Hz sinusoidal pitch rotation about the vertical axis. We are now testing vertical axis pitch VOR in darkness using electromagnetic search coils during 2-64°/sec peak velocity oscillations at 0.01 Hz. Sinusoidal functions with a positive offset term did not provide satisfactory fits to eye velocity during 0.01 Hz rotations above ~8°/sec. While higher head velocities resulted in greater downward slow phase velocities, the increase was not as great as would be predicted by the sum of drift and sinusoidal terms. Average peak upward slow phase eye velocity at 64°/sec head velocity was 43±14°/sec and average peak downward eye velocity was 96±6°/sec. Waveforms of slow phase eye velocities in response to high head velocities were markedly asymmetrical. Upward eye velocities rose steadily to the peak, while downward eye velocities appeared to saturate at a value near zero. These results suggest that in the 90° rolled position, upward but not downward slow phase vertical VOR is improved by a velocity storage mechanism as proposed by Matsuo and Cohen (Exp Brain Res 53:197, 1984). Supported by EY06485, EY07342, DC01559.
439.3 OPTOKINESTIC-VESTIBULAR INTERACTION IN PATIENTS WITH INCREASED VESTIBULAR-COULAR REFLEX (VOR) GAIN. E.W. Baloh1, J.M. Demer2. Reed Neurological Research Center, UCLA Sch of Med, Los Angeles, CA 90024-1769.

We studied optokinetic nystagmus (OKN) and visual-vestibular interaction in 5 patients with markedly elevated VOR gain. Approximately ±.3 normal across the frequency range of 0.01 - 1.6 Hz. Their lesions were localized to the vestibular nuclear complex on magnetic resonance imaging (MRI). All had impaired smooth pursuit and decreased initial slow phase velocity (SPV) of OKN SPY gradually built up over 25-30 sec. reaching normal values for low stimulus velocities (G 30 deg/sec). The initial SPV of optokinetic after-nystagmus (OKAN) was increased; but the reported decay of OKAN was normal. Fixation-suppression of the VOR was equally impaired whether the patients fixated on a surrounding optokinetic drum, or a light-emitting diode (LED) in the dark, or imagined an LED in the dark. These findings can be explained by a model that includes a velocity storage element and a variable gain element shared by the vestibular and optokinetic systems. Cerebellar lesions do not affect velocity storage but change the output of the variable gain element.

439.4 VESTIBULAR HABITUATION AND HUMAN REHABILITATION. K. Cohen1, C. Hatfield2, M. Kane3, L. Miller. Dept. of Otorhinolaryngology, Baylor College of Medicine, Houston, TX 77030, Program in Occupational Therapy, The University of Cincinnati College of Ohio, Toledo, OH 43699, Detroit Receiving Hospital, Detroit, MI 48201.

Repeatability vestibular experiments in many patients with unilateral peripheral vestibular lesions involves repetitive vestibular stimuli. Patients with unilateral peripheral vestibular lesions repeat habituation of velocity storage. We treated patients with unilateral peripheral vestibular lesions before and after receiving 12 biweekly therapy sessions involving repetitive vestibular stimuli. Subjects who had decreased vertigo and disequilibrium also had increased phase leads at low frequencies but not high frequencies. Therefore clinical compensation may involve low frequency habituation.

Supported by the Clayton Foundation for Research and the American Occupational Therapy Foundation.

439.5 CHRONOBIL VERTIGO: THE COMPARISON OF THE ACUTE AND CHRONIC FORMS. E.P. Primations1, E. Zaitman2. Boca Foundation. Ljubljan vestib. Klinika, FLUKA. 21027 Ljubljana, Yugoslavia. 197 Chronobil clean-uppers were studied in 1986 during the acute phase and in 1988 during the chronic phase. We compared the complaints by the patients. The most prominent features for both groups were complaints for vertigo accompanied by tinnitus and deafness, accompanied by nausea and vomiting in chronic phase. Headaches, tinnitus, phobias, cardiac pains and losses of consciousness were also added. If in the group of 1986 mostly the minor signs were recorded in the 1991 group there were mediately expressed signs of the vestibular dysfunction. The most significant for both groups were the data of the Uemura's test. The data obtained with cuedata were well expressed in the 1986 group and almost insignificant in the 1991 group. The acute and indicating tests data appeared to become significant (p<.05) in persons with distinctly expressed function change. The comparison of the CHRONOBIL vertigo with the drop attacks, Meniere's disease, vestibular neuritis, supratentorial lesion - shown that it had most prominent features, which were not common to the pathologies compared.

439.6 PERCEIVED PASSIVE BODY VELOCITY AND ACTIVE MOTOR HAND VELOCITY INTERACT WITH TEMPORAL FREQUENCY. M. Allen1,2, E. Katz3, C. Oman1,4, M. Gehring3.


The motion profile is a plot of object location as a function of time. For an object moving back and forth at constant speed the motion profile has a triangular waveform, with slop and amplitude that represent object velocity and path length respectively. Increments in the period of this waveform, motion profile temporal frequency (MPF), result in increases in perceived tactile (Katz et al., 1990) and visual (Katz et al., 1991) velocity, even when actual velocity is held constant. Here we studied MPF in the vestibular and motor systems.

VESTIBULAR: Four velocities and four angular path lengths served to define 16 rotational stimuli. Subjects discriminated the faster of two stimuli in a 2AFC paradigm. While on average they discriminated velocity correctly, 68% of the times if stimuli were of identical paths (slower stimulus had lower MPFF), when stimuli-pair had identical velocities, the shorter (higher MPFF) was judged faster (p<.05). MOTOR: Subjects were asked to move their right hand back and forth on a horizontal surface for a trial lasting about 30 sec. In darkness. At the beginning of each trial the subjects were instructed to move their hand faster than in the preceding trial. The data indicate that increases in velocity in successive trials was accompanied by reduction in path length and thus increasing MPFF. These findings indicate MPFF interaction with perceived velocity in the vestibular and motor systems.

439.7 PHYSIOLOGICAL RESPONSES TO MOTION SICKNESS DURING SINGLE-STOP VIDEOVESTIBULAR STIMULATION. B. D. Lawson1, E. Dilio1, M. A. Kassabiel1, J. Ventura1, and K. L. Lichten1. Ashland GrayWolf Spatial Orientation Laboratory, Brandeis Univ., Waltham, MA 02254. We studied how motion sickness (MS) develops (subjectively, by the stimulus) (EXP) and physical fitness (FIT) relate to heart rate (HR), blood pressure (BP), respiration rate (RR) and electroencephalogram (EEG) during movement stimulation. Subjects (n=14) rotated at 270 deg/sec for 30 sec, then stopped suddenly and opened their eyes. During a 30 sec period, they reported their MS and FIT. This procedure was repeated for 10 min while physiological responses were monitored. 10 min baseline and recovery periods were included. MS were performed on 4 time periods - baseline, early and late stimulation, and recovery. MS increased during stimulation (Friedman X2=22.5, p<.001), HR and BP showed a peak response (p<.005), EEG showed a peak response, and EEG did not change. Physiological measures did not distinguish high, medium and low MS groups. Subjects were ranked by NOG, EXP, EEG, FIT, and peak physiological response. MS correlated with EEG (.67), EEG (.59), EXP (.67), EEG (.67), EEG (.67), and EEG (.67). (All r-tailed p<.05). Thus, the physiological responses elicited were not strongly related to MS. Since MS, NOG, EXP, and also correlated to physiological response.


When assessing vestibular dysfunction, clinicians often rely more on subjective information (e.g., patient history) than on objective data (e.g., caloric test). As a result, vestibular dysfunction is usually characterized by gross descriptive classifications. We compared these broad classifications with more objective measures of passive vestibular gaze stabilization, and of torso self-movement. 10 dizzy patients were examined in the clinic, and their overall vestibular dysfunctions were classified. 10 (5 low, 5 high) ratings (I-2-3 scale: none; slight, moderate, etc.). Then, passive gaze stabilization was characterized as the seated subject was turned, during brief darkness, while looking at a target. Next, active head-torso movement was evaluated while the standing subject made maximal-velocity, but "comfortable", ±60°/s. In all cases, subjects were asked to evaluate their symptoms. We found that only ±60% of patients exhibited more gaze error, and slower torso movements, than normals. However, when gaze error was plotted against torso velocity, all abnormally-rated patients separated from the normals. Also, as a patient's rating became more abnormal, tendencies for gaze error to increase, and torso movements to slow, progressively widened this separation. Thus test results were closely related to the clinical ratings, perhaps because both vestibular and visual capabilities were assessed, the latter probably reflecting compensation to existing pathology. (Supported by Canadian MRC)
440.1

Between week 1 and 2 of postnatal life, there is a dramatic decrease in mean input resistance (Ri) of hypoglossal (presumptive genioglossal) motoneurons and a corresponding increase in Ri, which could be a doubling of total membrane surface area (A). We have quantitated the three-dimensional morphology of 25 genioglossal motoneurons at four different ages that have been labeled by intracellular injection of rhodamine in rat brainstem slices. There was no increase in Ri between 5-6 and 13-15 days; therefore, we must propose another mechanism to explain the decrease in Ri. However, the dendritic tree was not static during this period; there was a decrease in the number of terminals and the surface encompassing those terminals. This resulted from a loss of 7th and 8th order branches while there was an increase in mean segment length at the 2nd and 3rd order branches. This work was supported by NIH grant HD 22703.

440.2

The mean input resistance (Ri) of developing hypoglossal motoneurons undergoes a dramatic decrease between the first and second weeks of postnatal life. This alteration in membrane property could result from the growth of the cell membrane and/or a decrease in the specific membrane resistance (Rm). We have shown in these motoneurons that mean total area of membrane surface area (A) remains constant throughout this time period (see He et al). Thus, we set out to estimate the changes in Rm in these developing neurons. Intracellular recordings were made in hypoglossal (presumptive genioglossal) motoneurons from in vitro slices of the rat brainstem at various postnatal ages. Ri and membrane time constants were measured before the cells were labeled by intracellular injection of rhodamine. Ri was estimated for each cell (n=24) using an equation derived by De Robertis (1970). No difference was found between the mean Ri measured at 1-2 days (7116 Qcm²) and 5-6 days (7449 Qcm²) while there was a 47% decrease in the mean between 5-6 and 13-15 days (3969 Qcm²). After the decrease at 2 weeks, the mean Ri rose to 6192 Qcm² at 19-30 days as mean A expanded by 76% yielding only a modest decrease in Rm. We propose that the transient decrease in Ri results from i) an increase in number of leak channels in the postsynaptic cell and/or ii) a decrease in the number of presynaptic inputs on these neurons. These results suggest that there is a critical period during postnatal development when hypoglossal motoneurons are less excitable.

Supported by NIH grant HD 22703.
440.5 FAST MOTOR UNITS IN RAT GASTRÓNOMUS SHOWING EXTREMES IN FATIGUE RESISTANCE DO NOT DIFFER IN MOTOtàNEURONE PROPERTIES: PAIRED OBSERVATIONS FROM SINGLE EXPERIMENTS. P. Gardner* Physical Activity Sciences, University of Western Ontario, London, Mounta ine, Quebec, Canada H3C 3J7.

Differences in motoneuron (MN) membrane properties among motor unit types might be masked when grouped data from several experiments are analyzed. We consequently examined gastrocnemius motor units, paired from single experiments, that differed in type according to fatigue resistance and speed. Tidal MN of anesthetized Sprague-Dawley rats were impaled in situ experiments using conventional microelectrode techniques, and motoneuron motor units were identified from their contractile response to MN current injection. Type slow (S) units had twitch half-recovery times (RTw2) 2-8 ms, were completely fatigue resistant, and demonstrated no force sag at 25 Hz stimulation. In fast fatigue-resistant (FR) and fast fatigue-sensitive (FS) motor units pairs, twitch RTw2 were longer, twitches were weaker, and axon conduction velocities were faster, for FR. No differences in MN afterhyperpolarization (AHP) time course or amplitude, in rheobase, or input resistance, were found between FS and FR. S units had lowest rheobase, highest input resistance, highest AHP amplitude and longest AHP 1/2 decay time, but not always the lowest axon conduction velocity, when compared to FS units from the same experiments. Results show that MN properties, while showing distinct differences between fast and slow motor units, do not vary systematically among fast motor units which differ in fatigue resistance, in rat gastrocnemius. Supported by NSERC Canada.

440.7 MORPHOLOGY OF EXTERNAL URETHRAL SPHINCTER MOTOtàNEURONES AND LOCALIZATION OF PUTATIVE SPHINCTER INTERNEURONES IN THE RAT SPINAL CORD. V. Berthier*, M. B. D. Legape*, D. Bougrier, H., and J. T. Berthier. Department of Neurology and Behavior, SUNY at Stony Brook, NY 11794.

Spinal bladder/sphincter reflexes are: important for urinary continence, inhibited by supraspinal centers during micriturition, and involved in spinal cord injury induced bladder/sphincter dysynergia. However, little is known about the anatomical organization of these spinal reflexes.

In the present study, the dendritic morphology of external urethral sphincter (EUS) motoneurons and the distribution of putative EUS interneurons were examined using the retrograde tracer subunit (CTB), and the retrograde transneuronal tracer, wheat germ agglutinin (WGA), respectively. Adult male and female Sprague-Dawley rats were anesthetized, and either CTB (1%) or WGA (1%) was injected into the extrinsic urethra (0.5-3.0 h) into the EUS muscle. After a survival time of 2-5 days, spinal cords were removed and processed immunohistochemically to visualize labeled motoneurons and putative interneurons. CTB-labeled EUS motoneuron somata were located in the ipsilateral dorsolateral (DL) nucleus. Three major dendritic projections of these EUS motoneurons were observed: (1) rostrocaudally oriented dendrites projecting through the DL nucleus, (2) dorsolaterally oriented dendrites projecting into the lateral funiculus, and (3) medially oriented dendrites projecting toward the dorsomedial nucleus. WGA-labeled putative interneurons were localized to the ipsilateral L5 and L6 ventral horn dorsomedial to the DL nucleus. Supported by BNS-9111207 (WFC & JTE) and EY04587 (JTE).

440.9 AGED-LIKE ELECTRICAL PROPERTIES INDUCED BY ACRYLAMIDE IN TRIGEMINAL MOTOtàNEURONES OF THE AWAKE CAT. C. Wei*, S.S. Chieza, L.K. Enright, F.R. Marasli, and M.J. Chase. Dept. of Physiology, Dept. of Anatomy and Cell Biology, and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.032.

The effects of acrylamide on the electrical properties of motoneurons (MNs) include decreased conduction velocity (CV), increased input resistance (Rin), and axonal size in the mammalian optic nerve (F88; 180, 1987: 61,194, 1989). The mechanisms underlying the above-mentioned changes are not known. We sought to model some of these changes using acrylamide, a neurotoxin, in brainsmn MNs of the awake adult cat (methods in J. Neurophysiol. 58: 340, 1989). Jaw closer MNs were impaled with glass micropipettes (3M KC1, 5 M, their electrical properties were determined in vitro. Masseter MNs were also antidromically activated to evaluate their CV. Following the collection of these data control, administration of acrylamide between 0.01-1.5% caused a decrease in CV and Rin. Masseter MNs were also antidromically activated to evaluate their CV.

Comparisons between control and treated cells were made with one week while the cats continued to receive drug. Only cells with action potentials (AP) 55 nV were tested (AP 12 cm/s, 04 ms against the 25 acrylamide cat). Comparisons between control and treated cells were made with one week after the first tail t-tests (t=0.05). Significant changes occurred in the onset latency of the pharmacologically activated AP (13.6% increase) and in AP (40% increase). The Tm increased by 33% (p<0.05). There were no significant changes in the amplitudes of the AP or the duration of the afterhyperpolarization. While it is not known if acrylamide affects the same mechanisms as the aging process, this drug may provide a method for assessing contributing factors to the electrophysiological changes that are observed in aged MNs. Supported by AG04307 & NS00999.
441.1  HEAD AND TRUNK DYNAMICS OF YOUNG AND ELDERLY ADULTS DURING GAIT. R.A. Croswell* and R.A. Kestner. Dept. of Kinesiology, Univ. of IL at Urbana-Champaign, Dept. of Physical Therapy, Univ. of Illinois at Chicago.

Changes in gait with age have produced a more cautious and stable pattern (Winter et al., Phys Ther, 1990). Mechanisms underlying these changes may be found in intersegmental relationships. This study recorded the angular velocities of the head and trunk, and step cadence in four young adults (20-30 yo) and five elderly adults (60-70 yo) during self-paced, normal, slow, and fast walking. Neck velocity was calculated as the difference between head and trunk velocities. For the purpose of analysis, cadences were categorized at 90-120 steps/min for normal, >120 steps/min for fast, 90 steps/min for slow, and >120 steps/min for normal. (Normal cadences for the ELD subject fell between the slow and normal ranges). Proband frequencies of both the head and trunk velocity responses ranged from 1.2-2.3 Hz over all conditions for YA. Head frequencies ranged from 0.08-0.4 Hz, whereas trunk frequencies ranged from 1.2-2.0 Hz for conditions. Thus the head to trunk frequency ratio found in YA was not maintained in the ELD subjects, and time domain analysis of velocity revealed that the slow and normal ELG gait was similar to the YA slow gait in that the head, neck, and trunk velocity responses were in phase. A compensatory response was seen in the head, neck, and trunk of the YA normal and fast conditions; trunk flexion occurred with head and neck extension. In the ELD subject however, the fast cadence resulted in an uncoupling of the head, neck, and trunk where peak velocities of each segment occurred independently of the other segments. These data suggest that the ELD subject focused on keeping the head stable in order to better utilize vision. The coordinated phasing and the unity ratio of head to neck in the YA implies that the upper body is controlled as a coordinative structure. Uncoupling of these segments in the ELD subject show a decrease in the ability to control these segments as a unit.


We have studied the direction dependence of mean rectified surface EMG and motor unit recruitment while isometric forces were applied at the wrist. Previous studies have shown that recruitment thresholds of motor units for forces in various directions can be fitted by a single straight line or by several line segments (van Zuylen et al., J. Neurophysiol. 60, 1988). In a number of muscles, several subpopulations of motor units have been found, each with different motor unit recruitment characteristics (ter Haar Romeny et al., Exp. Neurol. vol. 85, 1984). Similarly, surface EMG for forces in various directions may be fit by a circle for many muscles (Miller et al. Exp. Brain Res. Series, 22, 1992), while for some muscles, more complex interpopulations functions were required (Flanders and Schlochter, J. Neurophysiol. 90, 1990). The aim of our study was to examine the relation between motor-unit activity and surface EMG.

Recruitment contributes primarily to EMG amplitude, while rate modulation affects its spectrum. The effect on the direction dependence of EMG, of these parameters and the inhomogeneous distribution of motor units, has not been tested directly. We have simultaneously measured surface EMG and intra-muscular motor unit activity from several arm muscles while human subjects exerted isometric force in many directions within a horizontal plane. In most cases, the EMG directionality could be fitted with a circle, and that of motor unit recruitment by a straight line. The orientation of the EMG response was generally consistent with that of the motor units. The few cases of disparity between EMG and motor unit orientation may be explained by sub-populations of motor units within a given muscle, each having a different orientation.

441.5  SINGLE-TRIAL ESTIMATION OF THE MECHANICAL PROPERTIES OF THE ELBOW JOINT DURING A MOTION TRACKING TASK. Y. Xu, J.M. Hollander* and J.W. Hunter. Department of Biomedical Engineering, McGill University, Montreal, Quebec, Canada H3A 2B4.

To determine the time-varying dynamic stiffness parameters (stiffness, viscosity, softness) of the elbow joint during movement, an ensemble averaging method must ordi- narily be used (Bennett et al., Exp.Brain Res., 1992). Because of the many trials tested in ensemble methods and the difficulties in aligning the movements, more recently we have been investigating how well time-varying dynamic stiffness parameters may be inferred from a single movement trial. To this end, we have proposed a tracking method based on exponentially weighted least squares (Xu et al., Proc. 13th DBBS Conf., 1990). In this method, the dynamic stiffness parameters must vary slowly for the tracking method to work properly, and movements must necessarily be slow. We have found that subjects have some difficulty in making smooth slow mo- tions, when the only constant is to provide targets, the smoothness is of course a good measure of the smooth- ness can be adversely affecting the smoothing. In the present study, we seek to improve trajectory smoothness by a mechanical aid: an artificial elbow joint and fore- arm which subjects can use. In our experiments, the artificial forearm is placed next to and parallel to the subject's arm, and the subject is instructed to track a point in the artificial forearm at the wrist level. The target movement spans 60 degrees in the vertical plane, starting at 90 degrees position and then extending. The movement is very slow, 12 degs/s. There are five phases in each trial, three post- target phases divided by two movement phases. Pseudorandom binary force perturba- tions are applied to the wrist by an isotropic system (Xu et al. IEEE Trans. Biomedical Eng., 38, 1991, pp.1111-1122), and we record force, wrist position and target pos- ition. Our results indicate that the stiffness parameter is lower during movement phas- es than in phases. However, the parameters do not have line- ar trend. This research is supported by the Medical Research Council of Canada.
441.7

EFFECTS OF RADIAL NERVE STIMULATION AND ANAESTHESIA ON INDEX FINGER MOVEMENT CONTROL. W.G. Darling, K.A. Cole, S. Ellis, C. Steyers. Dept. of Exercise Science, University of Iowa, Iowa City, IA 52242.

The role of cutaneous and joint afferents in index finger movement control has received little study, although reports from patients undergoing surgery indicate that finger kinesthesia is strongly affected by hand anaesthesia (Moberg 1983). We report that the EMGs and kinematics of isolated 3-joint index finger movements during non-noxious radial nerve stimulation and anaesthesia of the index finger determine whether control of its motion is affected by altered cutaneous and joint afferent sensations. EMGs were recorded using indwelling hooked wire electrodes inserted into 4-5 muscles of index finger in 10 subjects. Movements were recorded optoelectronically with infrared emitting diodes placed over the base of the 2nd metacarpal and the centers of the 3 joints. Subjects performed 4 different types of discriminative tasks (with vision permitted) that involved flexion or extension of all 3 joints and flexion or extension at the interphalangeal (IP) joints while the metacarpophalangeal (MP) joint was voluntary held in a fixed position. Kinematic data showed no consistent changes in movements as a result of radial nerve stimulation (train of 6 pulses at 30 Hz beginning near movement onset), however anaesthesia of the index caused decrements in movement velocities and amplitudes. EMG analysis indicated increased cocontraction of antagonists during anaesthesia and some effects on muscle activity as a result of radial nerve stimulation. Thus, these data suggest that cutaneous and joint afferent information plays a relatively small role in index finger movement control in comparison to central programs and, possibly, muscle propriospinal sensations at least when vision is allowed. supported by NIH grant AR40217

441.9


In recent experiments on the specialized frog (Rinella, Massa-Ivuli and Glister, Science, 235, 1992) we have found that the focal microstimulation of a site in the premotor layer of the lumbar gray matter results in a field of forces acting on the frog's ankle and converging to a single equilibrium position. These experiments suggest that the internuclear circuits in the spinal cord are organized into a set of control modules that "store" a few limb postures in the form of output fields associated with the postural points. The goal of our present investigation is to understand how such posture modules can be combined by the CNS for generating and representing a wider repertoire of motor behaviors including, but not limited to a large number of postures. In a theoretical study we have found that the vector summation of the convergent force fields generated by a set of 2 modules is a powerful mechanism for generating movements by combining a variety of control patterns. The most crucial issue regarding this computational approach is whether or not the fields generated by each of the 2 modules are actually combined by the CNS according to a principle as simple as vectorial summation. To address this issue we have considered the force fields evoked by the microstimulation of 2 distinct sites in the spinal cord. First, we considered the fields elicited by the stimulation of each site, separately. Then, we computed the vector sum of these 2 fields with the field obtained by stimulating the 2 sites simultaneously. Our current evidence indicates that the simultaneous activation of 2 internuclear sites generates a field of forces that corresponds to the vector sum of the fields generated by the stimulated site. However, we also found that the simultaneous stimulation of 2 sites within the affected fibers may result in a field that differs significantly from the vector summation of the fields obtained at each site. For example, we have observed "winner-take-all" responses to double stimulation of different systems. These results suggest the vector superposition of the motor output is a specific feature implemented by the internuclear circuits in the frog's spinal cord.

This work was supported by NIH grants NS05943 and AR36710, and ONR grant N00014-87-K-0732.

441.11


We present evidence for the view that the motor system takes advantage of the physical dynamics of the limb system during rhythmic movements. In particular, we tested the hypothesis that subjects choose a preferred rate of force generation which switches among the resonant frequencies of the physical system. Human subjects were asked to oscillate their forearms in the vertical plane at their preferred frequency under conditions of fixed mass and external force loading. They were also asked to oscillate at frequencies above and below the preferred frequencies by synchronizing their movements to a metronome. These trials were performed in order to generate a phase transfer function under external force loading condition which relates the phase between the displacement of the forearm and the torque generated by the biceps and triceps as a function of frequency. This phase transfer function was used to estimate the resonant frequencies to compute the stiffness of the joint (Viviani et al., J. Physi., Paris, 72, 45-58, 1978).

The results demonstrate that the preferred frequency corresponds to the resonant frequency of the muscle-limb system under the experimental conditions, a strategy that minimizes energy expenditure. This agrees with other studies which suggest but do not directly show that the preferred rate of oscillation corresponds to the natural frequency of the muscle-limb and elastic joint (Kugler & Turvey, 1987; Holt et al., Hum. Mov. Sci., 9, 1990).

We also show that the internal joint stiffness remains relatively consistent across added mass conditions but is modulated so as to match the impedance of the external springs, a strategy that maximizes the elastic energy stored in the joint (Goldfield et al., J. Biomech., 29, 1995).

441.12

CONVERGENCE OF EXCITATORY INPUTS FROM THE CENTRAL GRAY MATTER VOCAL CENTER AND INFERIOR COLICULUS TO A SINGLE FIBRILAR NEURON IN THE RAT. Y. YAJIMA* and Y. HAYASHI. Dept. of Physiology, Kyoto College of Med., Nishinomiya, Hyogo 663, Japan.

Two distinct phases of vocal communication behavior in animals, sound production and sound hearing, are assumed to be integrated in the brain. Present study was conducted to know an interface mechanism for vocal communication in rat brain. Under Ether anaesthesia a bipolar electrode was implanted stereotaxically into the ipsilateral central gray matter (PAG) vocal center using audible vocal sound yielded by pulse train stimulation as a guide. Under alpha-chloralose urethane anaesthesia the other electrode was placed in the contralateral inferior colliculus (IC) on the basis of auditory field potentials evoked by acoustic stimuli presented at an either ear. A grass micropipette filled with Fast green dye was introduced into the midline dorsal medulla to search for neurons activated by electrical stimulation of PAG and IC. Sixty-four fibrillar neurons sampled so far responded exclusively to PAG and 37 to IC stimulation (Non-convergent cells). Seventeen reticular neurons, on the other hand, were activated by electrical stimulation of both PAG and IC (Convergent cells). In a majority of cells, responses to PAG stimulation were augmented remarkably when PAG stimulation was preceded approximately by 5 msec to IC electrical stimulation or by 20 msec to noise burst stimulation, indicating the presence of modulatory convergence onto a single reticular cell. Histological verification revealed that most of convergent and non-convergent neurons were found in the ventromedial part of medulla.

Supported by the Natural Sciences and Engineering Research Council of Canada
Models of force control should be able to account for the both the mean form and variability of force trajectories. Whereas a number of studies have focused on the time course of force buildup, few have been given to the pattern of variation of the force-time function. In this study, we examine the mean and variability of three trajectories produced by squeezing a force transducer between the thumb and index fingers. Stimulation was at various times, as measured in milliseconds, in a random manner and direction (loading, unloading) of the force were varied. Step changes in force and force planes were recorded.

These data are used to test and extend the Parallel Force Unit Model of (Chen and Wing, 1991, Psych. Rev., 98, 268-294). In PFUM, force buildup and decline reflect the sum of a number of force units with variable onset times. The duration and number of the force units are controlled. This model reasonably well for the mean form and variability of short-latency responses. In this report, we extend our account for force trajectories under a variety of conditions. The model is elaborated to include antagonist "muscles" and additional motor unit-like properties.

A model based on the equilibrium point hypothesis of force control (Heldman, 1986, J. Mot. Behav., 18, 17-54) is also presented. According to this hypothesis, central commands regulate force by specifying the equilibrium position of the limb and the level of co-contraction of opposing muscles. Simulations are used to test the form of central commands underlying force generation. In addition, we examine how variation in these commands are related to variability of observed force trajectories. The equilibrium point model proposes a uniform account of uncorrelated and isometric movements.

Finally, these two models of force control are not mutually exclusive and we consider how they may be related.


The dual strategy hypothesis of motor control suggests that human elbow movements performed over short and different distances and/or with very light loads are generated, intensity is reduced when distance is increased. As such, the dual strategy hypothesis has been reformulated so that when movements of short distances and/or with very light loads are generated, intensity is reduced when distance is increased. It can thus be concluded that the CCN of the rat is a center of integration of sensory stimulation related to variability in observed force trajectories. The equilibrium point model proposes a uniform account of uncorrelated and isometric movements. Finally, these two models of force control are not mutually exclusive and we consider how they may be related.

THE INFERIOR OLIVARY NEURON AS AN ERROR DETECTOR IN THE MOVEMENT DISORDER INDUCED BY INTRACEREBROVENTRICAL INJECTION OF PROPIDIIUM IODIDE IN THE RAT. S. Chen and Z.-C. Peng (SPPON, IRBES), Institute of Anatomy, University of Verona, Italy.

Intracerebroventricular (ICV) injection of propidium iodide (PI) in the rat results in a movement disorder characterized by astagnia, ataxia and shakiness (Borges et al., Science 228, 246, 1985; Chan and Su, Brain Res 453, 379, 1989) and reduces c-Fos expression in discrete brain areas, including the inferior olivary complex and cerebellar nuclei (Chen and Bentivoglio, Soc Neurosci Abstr 17, 864, 1991). In the present study, in order to investigate whether the c-Fos expression was induced directly by ICV injection of PI or indirectly by the subsequent movement disorder, we studied the c-Fos expression induced by ICV injection of PI under urethane anesthesia, in which the animals do not show any movement disorder. PI (10 µl, 0.1% in saline) was injected under urethane anesthesia (1.2 g/kg, i.p.) into the lateral ventricle through a previously implanted cannula. The animals were sacrificed 3 hr after the PI injection. C-Fos immunoreactivity was distributed in the cerebellar nuclei as in the cases in which PI was injected without anesthesia. However, in contrast to the latter cases, in the inferior olivary complex only a few neurons in the medial nucleus were c-Fos immunoreactive. In the control injected saline no c-Fos immunoreactivity was observed in the cerebellar nuclei or in the inferior olivary complex. The present data, together with our previous findings, suggest that after the ICV injection of PI the activation of inferior olivary neurons results from the subsequent movement disorder, whereas the cerebellar nuclei are directly activated by PI administration. This supports the hypothesis that the inferior olivary neurons functions as an error detector or 'teacher' in motor control.

Single-joint discrete movements are likely produced by monotonic shifts in the equilibrium point (EP) of the system. On the other hand, oscillatory shifts of EP may underlie rhythmic movements. We tested the hypothesis that the two types of control signals can be superimposed to produce both movements simultaneously (Feldman 1989). Subjects showed flexion and extension movements about a target position indicated by a light on a horizontal surface. When the target position was shifted by 60°, the subjects made a fast movement to the new target while maintaining movement amplitudes. Subjects made isolated discrete movements from one target position to the other. Arm position was recorded on a Weisert system, and velocity and acceleration were calculated. EMG signals were recorded from the elbow flexors and two elbow extensors. The onset of discrete movement was associated with a deflection in the EMG signal and used as a synchronisation point for averaging of kinematic and EMG data. After averaging, the rhythmic component of the movement was eliminated and the residual discrete component was comparable to the isolated discrete movement made to the same target position. Fast discrete movements arose at any phase of the rhythmic movement, although some phases were preferred. Discrete movements were observed in combination with rhythmic ones displayed multi-phase EMG patterns and kinematics similar to those associated with isolated discrete movements. The data is consistent with the hypothesis of superimposition of control signals underlying discrete and rhythmic movements when they are performed simultaneously. However, the discrete component obtained by averaging displays a greater number of terminal oscillations and EMG bursts indicating that there is an integration of the discrete components at the executive biomechanical level. (Supported by NSERC Canada).

PERCEPTION OF VISCOSITY: SENSORY THRESHOLDS. J. W. Hunter and L. W. Hunter. School of Physical & Occupational Therapy and Dept. Biomedical Eng., McGill University, 3654 Drummond St., Montreal, Canada H3G 1Y5.

Sensory thresholds have been calculated for a number of different aspects of the human proprioceptive system including limb position, movement, force, and stiffness. Most of this research has focussed on differential thresholds, that is, the amount of a stimulus must be increased or decremented in order for the subject to discriminate that two stimuli differ. For the proprioceptive system, there is a remarkable amount of consistency in the differential thresholds measured for position, movement and force with the values varying between 6% and 10%. In contrast, subjects are much less consistent in discriminating changes in stiffness, for which the threshold is 23% (Jones & Hunter 1990). The objective of the present experiment was to extend these findings to an analysis of the sensitivity of subjects to changes in viscosity. This was measured using the matching procedure in which eleven subjects adjusted the viscosity of a servo-controlled linear motor connected to their left arm via a foot pedal containing an angular position encoder. Data was collected until it was perceived to be the same as that of the motor connected to their right arm. The servo-system was under computer (IBM 320) control and force and position were recorded from each motor via the encoders ranged from 2 N.m to 1024 N.m, and there were 8 repetitions of each of the 10 viscosities. The matching function obtained for viscosity was linear, with a very small deviation from linearity at the lowest viscosity (i.e. 2 N.m). The differential thresholds calculated for viscosity ranged from 83% at the lowest viscosity to 26% at the highest, and averaged 34% over the range of 22 to 1024 N.m. These values are almost 50% higher than those reported for stiffness, suggesting that the human proprioceptive system is less efficient at integrating force and velocity signals to perceive changes in viscosity, as compared to force and displacement cues for stiffness.

MODULATION OF THE RELATIVE ROLES OF SPIKING AND GRANDED SYNAPTIC TRANSMISSION BETWEEN GASTRIC PATTERN-GENERATING NEURONS OF THE LOBSTER GASTRIC MILL. T. G. Hunt* and A. L. Silverton, Department of Biological Science, Indiana University, Bloomington, IN 47401.

The purpose of this work is to develop a model of the lobster gastric mill CPG that is as simple as possible yet is biologically plausible, and which captures important network properties such as the emergence of patterns from network connectivity rather than endogenous cellular burst capability, continued pattern generation when cells are removed, and changes in the pattern after application of modulatory substances. The cell model, derived from the relaxation oscillator, has one fast current and a slow current that can be regulated by the combination of a fast inward and a slow outward current. It displays plateau potentials, postsynaptic inhibition, postsynaptic hyperpolarisation, and endogenous oscillations for suitable settings of two parameters. One parameter controls whether the fast current has a region of negative resistance and the other controls the gain of the slow current. When the region of negative resistance is present, endogenous oscillations occur. A network model of the complete gastric circuit was made, using a model of graded synaptic transmission but excluding spike-based transmission. For a wide range of parameter values, the model generates period-relationships that are approximately correct. Adjusting the gains of the slow currents in different cells causes small changes in phase relationships. A slow excitatory synapse causes a significant phase delay. Thus, a close match to in vitro recordings is possible. Also, some phase changes caused by neuromodulator application can be reproduced. The model network oscillates even when no component cell is an endogenous oscillator. When cells are killed, the network continues to generate a pattern provided at least one pair of reciprocal inhibitory cells remains. Current pulses were injected into cells in the network. Phase response curves and the limits of entrainment were computed for the model network, and compared with the biological data.

CIRCUITRY AND PATTERN GENERATION II

PROPERTY OF A RELAXATION-OSCILLATOR-BASED MODEL OF THE LOBSTER GASTRIC MILL CPG. W. R. Hunt and A. L. Silverton, Department of Biological Science, Indiana University, Bloomington, IN 47401.

The purpose of this work is to develop a model of the lobster gastric mill CPG that is as simple as possible yet is biologically plausible, and which captures important network properties such as the emergence of patterns from network connectivity rather than endogenous cellular burst capability, continued pattern generation when cells are removed, and changes in the pattern after application of modulatory substances. The cell model, derived from the relaxation oscillator, has one fast current and a slow current that can be regulated by the combination of a fast inward and a slow outward current. It displays plateau potentials, postsynaptic inhibition, postsynaptic hyperpolarisation, and endogenous oscillations for suitable settings of two parameters. One parameter controls whether the fast current has a region of negative resistance and the other controls the gain of the slow current. When the region of negative resistance is present, endogenous oscillations occur. A network model of the complete gastric circuit was made, using a model of graded synaptic transmission but excluding spike-based transmission. For a wide range of parameter values, the model generates period-relationships that are approximately correct. Adjusting the gains of the slow currents in different cells causes small changes in phase relationships. A slow excitatory synapse causes a significant phase delay. Thus, a close match to in vitro recordings is possible. Also, some phase changes caused by neuromodulator application can be reproduced. The model network oscillates even when no component cell is an endogenous oscillator. When cells are killed, the network continues to generate a pattern provided at least one pair of reciprocal inhibitory cells remains. Current pulses were injected into cells in the network. Phase response curves and the limits of entrainment were computed for the model network, and compared with the biological data.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 11, 1992
Dopamine (DA, 10^-5 M), serotonin (5HT, 10^-3 M) and octopamine (OA, 10^-2 M) each produce a unique pattern from the motor network in the stomatogastric ganglion of the lobster Panulirus interruptus. We are examining how amine modulate the pyloric rhythm. Here we describe amine effects on mixed graded chemical-electrical transmission between two different pairs of pyloric neurons. The AB and PD neurons are electrically coupled, in addition, the AB chemically inhibits the PD, while the LP and PY are reciprocally inhibitory. Modulation of synaptic rectification was examined in isolated neuron pairs in TTX-saline (20 C°). DA and 5HT reduced electrical coupling and enhanced chemical inhibition between the AB and PD. DA also reduced electrical coupling and enhanced the reciprocal chemical inhibition between the LP and PY. Oct enhanced while 5HT reduced PY chemical inhibition of the LP with little effect on electrical coupling. As a consequence of these differential effects on chemical and electrical transmission, the amine could reverse the sign of synaptic interactions between the cells. For example, depolarization of the LP depolarizes the PD in control conditions; this reverses to hyperpolarization during DA perfusion. In preparations where the AB to PD chemical synapse is weak and electrical coupling dominates, DA and 5HT change the synaptic interaction from weakly excitatory to strongly inhibitory. Modulation of mixed synaptic interactions contributes to the structure of the motor patterns produced by these amine. Supported by NNSA NS07859 and NIH grant NS17323.

We examined the I/O characteristics and amine modulation by dopamine (DA), serotonin and octopamine of 5 electrical synapses in the pyloric network in the STG of Panulirus interruptus. Some synapses (AB-PD and PD-PD) were non-rectifying, while the others (AB-PD, PD-PD, and LP-PY) all show significant rectification. The amine altered the strength of electrical coupling, but this varied with the different pairs of neurons and with the direction of current flow. For example, DA enhanced PD-PD but reduced AB-PD coupling. We modeled these results by an input resistance increase in the AB and decrease in the PD with no change in junctional resistance. DA causes these input resistance changes. For other synapses (such as DA reduction in AB-PD coupling), our model predicts that the amine reduces the junctional coupling in addition to altering cellular input resistance. Modulation of electrical coupling is a new mechanism by which amine modifies the pyloric network to generate multiple motor patterns. (NNSA NS07859 and NIH NS17323)

In the trichocerebral pyloric motor pattern, the pacemaker neurons inhibit the LP and PY neurons; these cells show different rates of postinhibitory rebound, causing them to fire at different phases in the cycle. Dopamine (0.1mM) enhances the LP and PY cell firing and causes them to fire at the same phase in their activity. This is explained in part by dopamine inhibition of the LP-PD and PY-PD synapses. Two additional mechanisms for phase advance are intrinsic to the post-synaptic LP and PY cells. 1) Conduction decrease of 1a. Both LP and PY express a transient K+ current (1a) that is selectively sensitive to 4-amino pyridine (4-AP). This current plays an important role in the rate of rebound after hyperpolarization in synaptically isolated LP and PY neurons. Both DA and 4-AP reduce the delay to first spike after 200 msec hyperpolarizing pulses, and increase the frequency of firing in response to tonic depolarization. Blockade of 1a by 4-AP occludes the effect of DA on the LP cell suggesting that reduction of 1a is the major action of DA in this cell. 2) 1b. The LP expression of 1b. The LP cell displays a Ca++ sensitive depolarizing sag during tonic hyperpolarization, caused by the hyperpolarization-activated inward current, 1b. DA enhances this sag. Specifically by shifting its voltage dependence for activation in the depolarizing direction. This effect is eliminated by low concentrations of Ca++, which eliminate 1b. This suggests that DA acts on the LP by both reducing 1a and enhancing 1b. We are now using voltage clamp of cultured LP and PY cells to try to directly measure dopamine modulation of these currents. Supported by NIH NS17323 and NS 25915.

442.6 INWARD CURRENTS UNDERLYING BURSTING IN CULTURED STOMATOGASTRIC NEURONS. G.G. Turrigian* and E. Marder, dept. of biology, Brandeis University, Waltham, MA 02254.
Stomatogastric (STG) neurons in situ express slow regenerative conductances that underlie bursting and plateau behavior. We use primary cultures of STG neurons from the spiny lobster Panulirus interruptus to identify two inward currents that contribute to these properties. STG neurons in culture switch from tonic firing to bursting in TEA. In most neurons these oscillations, as well as fast spikes, can be blocked by TTX. In other neurons TTX has little effect on oscillations; these oscillations are blocked by M+m++. Under two electrode voltage clamp we have identified two persistent inward currents, one that is blocked by TTX, and one that is blocked by M+m++. The TTX-sensitive current begins to activate above -40 mV, peaks at +20 mV, and has an extrapolated reversal potential of +20 mV. The M+m++-sensitive current activates above +40 mV, peaks at +20 mV, and reverses at +50 mV; the magnitude and reversal potential of this current depend on the external Ca++ concentration, suggesting that the current is carried by Ca++ ions. Most STG neurons have both inward currents, but their relative magnitudes, and thus the pharmacological sensitivity of bursting, vary from cell to cell. We are currently investigating whether the ratio of these conductances is a function of neuron type. Supported by NS-08971 to G.G.T. and BNS-9002951 to E.M.

Model neurons are useful in understanding the complex interactions between single currents in a cell, or between different cells within a network. To study the dynamics of both synaptic and intrinsic properties of neurons we connect a conductance-based model neuron to a real neural network. We use a DSP board and the MAXIM software package to run the model and control the input-output interface. The system allows the model neuron to run and respond in real time to artificial synapses created between real neurons and the model neuron. The model neuron was constructed using data from the LP neuron of the stomatogastric ganglion. We make reciprocal inhibitory connections between the model LP neuron and a biological PD neuron and study the effect of changes in both inputs on the LP neuron. Finally, we investigate the effects of changes in the strengths of the synapses between the model and the biological neuron. The results suggest that the model LP firing phase in the pyloric network depends critically on the characteristics of the synaptic inhibition coming from the LP neuron. The burst duration and the firing frequency of the LP model depends on Ih and IA. Supported by MH46742 and The Human Frontier Science Foundation.

442.8 Dynamic Clamp: A method to construct artificial synapses and assess the role of voltage-dependent and synaptic conductances. A.A. Sharp*, M. O'Neil, G. LeManson, L.F. Abbott and E. Marder. Dept. of Biology, Brandeis University, Waltham, MA 02254.
Conventional voltage-clamp techniques provide data describing how the conductances that shape neuronal excitability depend on voltage and time, but do not allow the investigator to determine the participation of individual currents in the dynamic behavior of a neuron. We have devised a method, called a dynamic clamp, that allows us to increase, decrease or alter conductances in neurons during their normal dynamic behavior. This method is used to study the effects of a given voltage-dependent or synaptic conductance on a cell's intrinsic behavior and the emergent properties of a network. It is also used to create new networks by generating artificial synapses.
To construct the dynamic clamp, an Axoclamp in DCC mode is used to monitor the cellular potential and to inject current. Based on this potential and a computational model describing the dynamics of the conductance under investigation a computer determines the current to be injected. When constructing synapses both the pre- and post-synaptic potentials are monitored. The investigator controls the relevant thresholds, reversal potentials and time constants. The investigator can create synapses between dissociated neurons in culture, in the intact stomatogastric ganglion and/or to modify the intrinsic properties of neurons within these networks. Supported by MH 46742 and NSF BNS 9009257.
**442.9**


The highly conserved crustacean cardioactive peptide (CCAP) activates the pyloric rhythm of the cerebrovascular nervous system in a dose-dependent manner with a threshold at 10^{-10}M. The number of spikes/burst in the lateral pyloric (LP) neuron, a major target, increases from 2-5 spikes/burst in control saline to >50 spikes/burst in 10^{-10} CCAP. This increase in spikes/burst is associated with the induction of plateau potentials in the LP neuron. Bath application of CCAP (>10^{-9}M) causes oscillations in the membrane potential of the pharmacologically isolated LP neuron. The phase of the oscillations can be reset with brief current pulses into the LP soma. This is responsible for the change in the LP duty cycle from 10% to over 40% of the phase in 10^{-9}M CCAP.

Simultaneous endoscopic observations of the valve between the gastric mill and the pyloric chamber and extracellular recordings of the major STG motor nerves in intact crabs suggest that changes in the LP neuron's activity dramatically alter the movements of this valve.

Supported by NS17813 (EM), GRF HE1118 (HGH) and Human Frontiers Science Program.
442.17 PATTERNED ELECTRICAL STIMULATION OF CULTURED NEUROAL PLATE E. B. Rhodes* and G. W. Gross, Dept. of Biological Sciences, University of North Texas, Denton, TX 76203.

Cultured neuronal networks derived from the embryonic mammalian spinal cord display spontaneous activity which is characterized by the temporal consolidation of spikes into bursts and a variable degree of burst synchrony across the network. The multimicroelectrode plate (MMEP) is a culture substrate incorporating an array of 64 point extracellular microelectrodes, provides a uniquely stable and interactive system for studying active neuronal cultures.

Networks of 50-100 neurons cultured on MMEEPs (from dissociated spinal cord tissue of 14-day mouse embryos, and activity was recorded after 20-35 days in culture. Monopolar and bipolar stimulation pulses (1-1.5 μsec) were applied to a 10Ω resistor in front of the preamplifier FET of one or more recording channels (average impedances @ 1kHz: 1.5MΩ electrode, 12MΩ amplifier input, 40Ω microphone-bath shunt).

Single pulses delivered to a single microelectrode can trigger network-wide burst responses. The threshold strength-duration relationship for this response can be fit by standard physiological curves, with a unique rheobase and chronaxie for each stimulation site.

This is the first demonstration of effective electrical stimulation of a cultured network via point extracellular electrodes, and opens several intriguing avenues for further research. These include training networks to exhibit novel spatio-temporal activity patterns with repeated patterned stimulation, testing the Hebb rule as the network level in both spike and burst domains by using spike- or burst-triggered stimulation to drive network activity, and developing a cultured network model of behavior.

Supported by grants from the Texas Advanced Technology Program and the Hillcrest Foundation of Dallas, Texas.

442.18 OPTICAL AND PHARMACOLOGICAL STUDIES OF PROPRIOSPINAL NEURONS INVOLVED IN RHYTHMIC MOTOR ACTIVITY IN THE EMBRYONIC CHICK SPINAL CORD. Stephen H. Poznanski and Michael O'Donnell, Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Our previous studies have identified a class of proprioceptive neurons whose axons travel in the ventrolateral funiculus of the spinal cord (VLF) and are involved in synchronizing motoronoeur discharge during rhythmic motor activity. The properties of these LT neurons were studied further in ES-10 chick embryos using optical and pharmacological methods. LT neurons were labeled retrogradely with a Ca indicator (Ca-green dextran), and were found on both sides of the cord following an unilateral LT injection. (ipsilateral cells were distributed dorsal and medial to the lateral motor column. The contralateral group was more discrete and was located in the medial part of the cord, near the central canal. During episodes of motor activity, both groups expressed synchronized, rhythmic fluorescence signals in phase with motor neuron discharge.

LT stimulation resulted in a brief synchronized discharge in flexor and extensor motor neurons followed by a more prolonged burst. The synchronized discharge and the prolonged potentials were abolished respectively by CNX (5 μM) and AP-5 (50 μM). Depolarizing potentials persisting in the presence of CNX (1-2 mM) were blocked by strychnine (50 μM) or bicuculline (100 μM). LT evoked potentials are not mediated by electrical coupling or by chloride transmission because they were blocked by CNX and were unchanged during bath application of high K medium. These findings indicate that LT axons have both excitatory and inhibitory connections with motoneurons. In addition to their role in segmental coordination they may also participate in cortically mediated inhibition that underlies the alternation of flexor and extensor motoneurons.

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SELECTIVE IMPAIRMENT OF DELAYED NON-MATCHING-TO-SAMPLE (DNMTS) FOLLOWING FRONTAL CORTICAL LESIONS IN THE RAT. L. M. Harrison* and R. G. Nair. University of New Hampshire, Durham, NH 03824

Two experiments were conducted to determine the effects of lesions of the lateral internal medullary lamina of thalamus (L-IML) and of frontal cortex on three measures of spatial memory: DNMTS, radial arm maze (RAM), and serial reversal learning (SRL). In Exp. 1, RAM was trained prior to surgery. While both RAM and SRL were disrupted by lesions of the L-IML, neither task was affected by destruction of frontal cortical areas along the medial wall (MW) or dorsal to the rhinal sulcus (RS). In Exp. 2, DNMTS was trained prior to surgery. Rats with lesions of MW or RS showed impairments comparable to those seen following L-IML lesions. Anatomical studies were conducted using wheat germ agglutinin - horseradish peroxidase (WGA-HRP) to map out projections of the mediadorsal nucleus (MDm) of thalamus in controls and in animals with RS and MW lesions. These analyses showed that the RS and MW lesions produced near-complete destruction of thalamo-cortical and thalamo-cerebellar projections to cortex, and that neither lesion (MW or RS) disrupted pathways leading from the MDm to the other frontal target area (RS or MW).

443.3


In a critical test of the configural association theory of hippocampal function, Sutherland and Rudy (Psychology, 17:129, 1989) reported that rats with neurotoxic (cochicine + kainic acid (COL + KA) lesions of the hippocampus were unable to learn a negative patterning problem. In the present experiment, we extended this neurotoxic model to rats with either ibotenic acid (IBO) or COL + KA lesions of hippocampus, together with controls, were trained on a negative patterning discrimination where responding in the presence of either a tone or light was reinforced while responding when a tone or light were presented in compound was nonreinforced. Following acquisition, transfer trials were given to see if the negative patterning problem had been learned using configural associations. The results indicate that (1) rats in both COL + KA and IBO hippocampal groups learned the negative patterning discrimination, (2) COL + KA rats responded at higher rates, and (3) all groups learned the problem using a configural solution. Since neither type of hippocampal lesion impaired the ability of rats to solve the negative patterning problem, and transfer tests indicated that the groups learned the problem using configural associations, these results fail to support the configural association theory.

444.4

EFFECTS OF PERIPHERAL CORTEX AND MEDIAL EXTRASTRIATE VISUAL CORTEX LESIONS ON MEMORY ASSOCIATED WITH AN OBSESSION CONTINUOUS RECOGNITION TASK. A. Backman and H. Backman. Dept. Psychol., Univ. of Utah, Salt Lake City, Utah 84112.

The perirhinal cortex is a major convergence site for afferents from the neocortex. Thus, it may play an important role in linking the neocortex to allocortical areas thought to be involved in recognition memory, including the hippocampus. In order to test this possibility, we lesioned the medial extrastriate visual cortex and the perirhinal cortex, and studied the effects of these lesions on rats trained to discriminate two odors. The training procedure involved the sequential presentation of eight novel objects, and four repeated objects (chosen from the eight) within a session. These rats were divided into 120 different 3-dimensional objects in various shapes, sizes, textures and degrees of brightness. Repeated objects had lags ranging from 0 to 4 (from 0 to 4 different objects were presented between the first and the last presentation). An object was presented on one side of a long table divided in half by an opaque Plexiglas guillotine door, and the latency between opening the door and the rat moving the object was measured. On this measure of stimulus presentation, the rats scored better on the visual compared to the olfactory task, and only the initial presentation of an object resulted in food reinforcement. Rats were assigned to groups and received training upon reaching criterion performance. Prior to surgery, lags were significantly longer on repeated presentations than on non-repeated presentations for all rats. There was no deficit following sham lesions or medial extrastriate visual lesions, whereas post-surgery performance was significantly worse than pre-surgery performance for the perirhinal group. Thus, the perirhinal cortex plays an important role in memory associated with this object continuous recognition task, whereas the medial extrastriate visual cortex does not.

444.5


Combined loss of hippocampal acetylcholine (ACh) and serotonin (5-HT) occurs in Alzheimer's disease and may interact to impair memory. To examine this hypothesis, we tested rats pretrained in a 12 arm radial maze for the separate and combined effects of NMDA induced lesions of medial septal neurons and 5,7-DHT induced lesions of the fimbria/ fornix plus cingulate gyrus, on reference (RMm) and working (WMm) memory. NMDA and 5,7-DHT reduced hippocampal acetylcholine and 5-HT in 48% and 82% of control, respectively. Neither treatment alone nor combination affected WMm. The 5,7-DHT treatment did not affect WMm. NMDA caused an increase in WMm errors. The combined treatment and 5,7-DHT did not differ from the NMDA-only effect on early postoperative WMm. However, the combined lesion did appear to retard the post-operative recovery of WMm that was observed for the NMDA-only treated rats. In Alzheimer's disease, hippocampal 5-HT deficits may prevent compensation for the disruption of working memory that is caused by loss of hippocampal ACh function.

Rats trained to run a straight alley for a large food reward display sharply decreased latencies when shifted to a small reward. This behavioral phenomena, minimal to the Crews effect (1942) or behavioral contrast, is often interpreted as an aversive emotional reaction to a reduction in reward magnitude. The behavioral contrast paradigm has been used extensively in studies examining analgesic effects of drugs. The present study used the behavioral contrast paradigm to examine the role of the amygdala in memory for a reduction in reward magnitude. Male Sprague-Dawley rats (175-200g) were implanted with bilateral electrolytic lesions and trained to run a 10 arm alley (6 trials/day) either in or on 1 mg food pellets. On day ten of training, half the animals in the high reward group were shifted to a one pellet reward. Immediately following trial training, the animals received an intra-amygdala injection of either a 18 lidocaine solution or phosphate buffer (0.5 μl/bed). Shifted training continued for two more days and no further injections were given. Shifted animals that received a buffer injection displayed a characteristic increase in escape latencies on the second day of shifted training. In contrast, animals that received lidocaine injection showed no increase in latency on either the 2nd or 3rd day of shifted training. The findings indicate that post-training inactivation of the amygdala attenuates behavioral contrast, suggesting that the amygdala is involved in regulating memory for reduction in reward magnitude.

Supported by NSF fellowship RCD-9054728 (JS), PHS grant 1 F32 NS08973-01 (MOP) and PHS MH12526 (NIMH and NIDA) and ONR N00014-90-J-0167 (ILM).


The purpose of the present experiments was to investigate the role of the amygdala in anxiety, using two different, pharmacologically validated animal models of anxiety: the elevated plus-maze test and the shock-probe burying test. Experiment 1 showed that animals with complete amygdaloid lesions made significantly more contacts with the electrified probe than sham-lesioned controls, but were not significantly different from controls in the duration they buried the probe or the amount they explored the open arms of the plus-maze. Experiment 2 replicated these results, and showed that rats with larger unilateral and with smaller bilateral lesions made more contacts with the electrified probe than rats with smaller amygdaloid lesions. Experiment 3 showed that amygdaloid lesions did not alter the ability of diazepam to increase open-arm activity in the plus-maze test or to decrease burying behavior in the shock-probe test. Taken together, these results suggest that 1) the amygdala does not mediate the anxiolytic effects of diazepam in these tests, and 2) the amygdala may be involved in the inhibition of some fears [e.g., fear of an electrified probe] but not others [e.g., fear of an elevated, open arm].


Olfactory-guided learning in rodents can serve as a useful model system for the study of the neurobiological bases of higher order processes in learning and memory. The present experiment involved the development of an odor-guided paired-associate task (PA) analogous to the verbal PA task frequently used to assess human memory.

In the present version of the PA task, rats are trained to sniff at an odor port while two different odors are presented in succession, separated by a brief blank interval. If the stimulus sequence involves any one of four arbitrarily assigned paired associate (PA) odors, the rat is rewarded for responding at a nearby water port. Alternatively, if the sequence consists of any other combination of the same eight odor ("matchup") the subject must withhold a response. In addition, the rat must undergo a 15 second delay between presentation of "non-associative" trials involving one of the eight odors paired with one of four odors from a second set; these odor sequences are never rewarded.

Last rats learn to discriminate the non-relational trials rapidly, and more gradually differentiate mispairings from paired associates. Rats with bilateral ablation of the perirhinal-entorhinal cortex were profoundly impaired in learning to distinguish mispairings from paired associates. However, the same isolated PA showed only a mild and transient deficit on non-relational trials. These results support the hypothesis that the hippocampal system is critical to PA learning across species and, more generally, that this system supports memory representations based on arbitrary relationships among perceptually independent cues across stimulus modalities.


443.10 MEMORY-BASED LEARNING DEFICITS PERSIST INTO ADULTHOOD AFTER X-IRRADIATION-INDUCED HIPPOCAMPAL GRANULE-CELL HYPOPLASIA IN EARLY INFANCY. N. I. Lobaukh*, J. L. Diaz-Granados, P. L. Greene & A. A. Amald. Department of Psychology & Institute for Neuroscience, University of Texas, Austin, TX, 78712.

Our laboratory has shown that electrolytic hippocampal lesions in infant rats (Lobaugh, et al., Behav. Neurosci., 103:1139, 1989), postnatal exposure to ethanol (Greene, et al., Behav. Neurosci., 106:51, 1992), and early postnatal hippocampal x-irradiation (Diaz-Granados, et al., Soc. Neurosci. Abst., vol. 16, 1990) all disrupt the acquisition of patterned (single) alternation (PA), a form of memory-based learning, in infant rats. After x-irradiation exposure, pups tested at 17-18 days of age showed a severe deficit in PA with a 60-s interval intertrial (ITI) but not a 30-s ITI. In the present experiment, we examined the effect of early postnatal exposure to x-irradiation on memory-based learning in adult rats at 60-85 days of age. The irradiated animals showed a significant deficit in PA learning at a 60-s ITI when compared to normals and sham controls. The specific reductions of hippocampal dentate granule cells at day 86 were comparable to the reductions in the younger animals of our earlier result. These results suggest that early insult to hippocampal granule-cells by x-irradiation results in lasting memorial and morphological deficits. Supported by NSF grant BNS-8609877.

443.11 A PRELIMINARY ANALYSIS OF THE BEHAVIORAL EFFECTS OF LESIONS OF THE SUPERIOR CEREBELLAR PEDUNCLE IN R.C. Leasure and D. J. Kaelin. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

A series of tests assessed the behavior of rats with knife-cut lesions of the superior cerebellar peduncle as compared with sham operated controls. In the first of two 20-min sessions in a brightly illuminated open field the lesioned rats were significantly more active than controls. This difference disappeared at the second session 24 hr later. In tests of the acoustic startle response the lesioned rats were significantly more responsive than controls. They showed significantly more robust within-session response decrements than controls. Both groups showed significant response decrements over days, i.e., long-term habituation, but the decrements appeared somewhat more variable in the lesioned animals. On the balance-beam test of motor stability, there was no significant difference between the groups. Neurological examinations revealed some deficit in the lesioned animals in head-placing. Overall we were most impressed with the subtle nature of the behavioral effects in the lesioned animals. Histological analysis showed significant, near-complete bilateral section of the superior peduncle in 6 of the 7 lesioned rats.


Previously we have shown that PTD treatment in rats impairs working memory and produces consistent lesions of the internal medullary lamina (IML) surrounding MDn. Examination of Fink-Heimer stained tissue has shown widespread layer IV cortical denervation, including all areas of frontal cortex. To determine the extent of frontal cortical denervation, we implanted WGA-HRP bilaterally into 10 rats recovered from PTD, aimed at areas of MDn typically spared by this treatment. Rats with IML lesions and spared cortical effects of MDn showed retrograde transport of WGA-HRP to frontal cortex. Rats with near-complete MDn lesions did not. These findings indicate that areas of MDn spared in PTD rats remain connected to frontal cortex.
MK-801 IMPAIRES LEARNING BUT NOT RETENTION FOR SERIAL PATTERNS IN RATS. J.D. Rowan* and S.B. Fountain. Department of Psychology, Kent State University, Kent, OH 44242.

MK-801 is a N-methyl-D-aspartate antagonist that has been found to impair learning by increasing the effects of MK-801 exposure on learning and retention of serial patterns were examined. Rats were trained in a serial pattern learning task to anticipate elements of one of two patterns. The task required rats to anticipate the correct sequence of leverpresses in an array of levers mounted on the walls of an octagonal shaped operant chamber. Both patterns were equally structured, but one pattern contained a violation of pattern structure. In the first phase of the experiment, male hooded rats were exposed to i.p. injection 30 minutes prior to testing each day during acquisition. Rats exposed to MK-801 during acquisition were significantly impaired in learning the patterns compared to saline injected controls. The rats showed impairments primarily in learning the pattern containing the violation of pattern structure. In the second phase of the experiment, saline control rats from the first phase that had already learned their patterns were injected with 0.0625 mg/kg MK-801 to assess its effects on retention of the serial patterns. Compared to saline controls, rats exposed to MK-801 were not significantly impaired in retention. These results support the view that MK-801 impairs the acquisition of new serial patterns but does not affect retention of previously learned serial pattern information. (Supported by NIH BRSG 5078R7208 and NIMH MH48402.)

DIFFERENTIAL BEHAVIORAL EFFECTS OF SINGLE OR COMBINED LESIONS TO THE INFRA- AND SUPRACALLOSAL, SEPTO-HIPPOCAMPAL PATHWAYS IN THE RAT: WORKING MEMORY DEFICITS ARE NOT AMELIORATED BY OCETROMINI H PILOCARPINE. P.L. Green, J.C. Cassel, C. Kelche, H. Jeltch & B.E. Wib*, N.B.C. UPR 419 du CNRS, Université Louis Pasteur, 67000 Strasbourg (France).

Female Long-Evans rats sustained single electrolytic infrastrucal (group IN, n=13) or aspirative supracallosal (group SU, n=12), or combined lesions (group INSU, n=12) of the septo-hippocampal pathways at 90 days of age. Sham-operated animals (group SH, n=13) served as controls. Infra- and supracallosal pathways were chosen because of their differential content of cholinergic fibers. There was an infrastructural lesion-induced groups IN and INSU) increase in open-field and home-cage activity, and decrease in spontaneous alternation and habituation to novel stimuli 15 days post-lesion. Of these effects, only increased open-field activity and decreased spontaneous alternation were again observed at 75 days post-lesion. Performance in an 8-arm radial-maze task was impaired as well, again only in groups IN and INSU. These differences in radial-maze performance between groups became more robust with a one-minute delay imposed between the 4th and 5th choices. Ocetromine (0.1 & 0.03 mg/kg) and Pilocarpine (1.0 & 0.32 mg/kg) were ineffective at improving the performance of impaired animals either with or without a delay between the 4th and 5th choices in the radial-maze task. There were no differences between groups SH and SU among the behaviors observed in the present study. The results are discussed in relation to the importance of septo-hippocampal cholinergic pathways in working memory and in terms of the possible relevance of interactions between acetylcholine and other neurotransmitters in memory function.

RF LESIONS OF THE LATERAL INTERNAL MEDULLARY LAMINA (L-IML) OF THALAMUS IN THE RAT INCREASE THE RATE OF TEMPORAL DECAY OF A DELAYED NON-MATCHING TO SAMPLE TASK. E.E. Kylvahan* and R.G. Mair. Dept. of Psychology, University of New Hampshire, Durham, NH 03824.

36 rats were matched for performance on a DNMTS task and then randomly assigned to control or to 1 of 3 lesion groups: L-IML, mediiodorsal nucleus of thalamus (MDN), or fornix (Fmx). After recovery, rats were trained for laterality on DNMTS at 6 delays from 1.8 to 8.8 s. L-IML animals performed significantly worse than all other groups, exhibiting significantly faster rates of decay up to 6.3 s (when their performances reached chance level). MDN and Fmx rats were not significantly impaired on DNMTS. All 3 lesion groups were significantly impaired compared to controls when subsequently trained on an 8 arm radial maze task.

PUTATION OF ODOR AVERSION BY TASTE IS DIFFERENTIALLY AFFECTED BY 6-OHDA OR QuISUlate INJECTIONS. J. Fernández-Ruiz, R. Guzman, M.I. Mirgoja, E. Berndez-Battan, and P. Druet-Ronc, Departamento de Fisiología, Facultad de Medicina y Instituto de Fisiología Celular, UNAM, 04510 México DF.

It has been demonstrated that Parkinson's disease patients have an olfactory deficit. One possible cause may be that dopamine depletion of the olfactory structures leads to an olfactory dysfunction. To test this hypothesis we submitted two control groups, three quisqualate and three bilateral 6-OHDA lesioned rat's groups to a modified potentiation of odor aversion by taste paradigm. The regions tested were the insular cortex, the amygdala and the hippocampus dorsalis. At the end of the experiment the 6-OHDA brains were prepared for HPLC. The behavioral results showed that both the Injection of 6-OHDA or quisquulate in the insular cortex produce the disruption of taste, but not odor aversions. On the other hand, the lesion of the amygdala with 6-OHDA or quisquulate, caused the disruption of the potentiated odor, but not the taste aversions. Finally, the quisqualate, but not the 6-OHDA lesions in the hippocampus dorsalis produced a disruption of both taste and potentiated odor aversions. The HPLC showed a decrease in catecholaminergic contents of the lesioned sites. These results indicate that the Injection of 6-OHDA in the amygdal region, but not in dorsal hippocampus nor in the insular cortex, produced a severe olfactory deficits. Supported by FIRESIN and DGAPA-IN-204689.
CORTICAL CYTOARCHITECTURAL ABNORMALITIES CORRELATE WITH COGNITIVE DEFICITS IN MICE. J.C. Holzmann*, J.L. Sweitzer, E. Backman, G. Older; Kennedy Krieger Institute, Baltimore, MD; Wellesley College, Wellesley, MA; Harvard-MGH, Boston, MA
In neonatal mice, lesions of the basal forebrain (Bf) projecions to cortex in rats elicit profound cholinergic denervation which lead to persistent changes in cortical connectivity and cytoarchitecture. Because these cytoarchitectural abnormalities manifest morphological changes observed in human mental retardation, this is a model for developmental disabilities. We have shown previously that Bf lesions in neonatal rats increased motor activity, decreased anxiety and increased swim maze latencies in adult mice. The purpose of this study is to determine whether there are correlations between histological abnormalities and behavioral impairments in these mice. 25 Balb/C by C57Bl/6 mice received lesions to the Bf (24±4 hours after birth. Behavioral testing began at 8 weeks and included assessment of motor activity, retention of passive avoidance task) and swimming task. Following behavioral testing, 9 mice were killed for Nissl and AChE histochemistry. Morphological abnormalities were evaluated and scored. Lesions varied in size, anterior/posterior and medial/lateral extent of damage and severity of altering cortical abnormalities. Independent of lesion location and size, cortical AChE intensity and distribution were comparable to controls. Motor activity did not correlate with passive avoidance or swim maze latencies. Additionally, lesion location, size and cortical cytoarchitectural abnormalities did not correlate with motor activity or passive avoidance retention latencies. Cortical histology did, however, correlate with swim maze latency at 60; p = 0.0003), i.e., severely abnormal cortical morphology predicted high swim maze latencies. These data indicate that cortical cytoarchitectural abnormalities resulting from Bf lesions in neonates correlate with impaired learning, but not stress-related measures in adult mice. Thus in this lesion model and, by extrapolation, in neonatal retardation, mutant changes in the cortex which result from ontogenic abnormalities could lead to behavioral changes later in life.

INTERACTIONS BETWEEN EMOTION AND MEMORY FORMATION: A PROPOSED HYPOTHESIS. D. Galey*, Laboratoire de Neurosciences comportementales et cognitives, CNRS URA 339, Université Bordeaux I, avenue des Facultés, Talence Cedex, France.
Using two inbred strains of mice (BALB/c and C57Bl/6), we have attempted to elucidate the relationships between memory processes and the modulation of activity of the septo-hippocampal cholinergic pathway.

At first, we have shown in BALB/c mice that medial septal stimulation (30 nA, 300 Hz) applied 30 sec after the partial acquisition of an operant lever press condition increased subsequent retention performance 24 hours later with a temporal gradient of the stimulation effect less than 30 sec before. This result suggested that the stimulation improved consolidation processes. In these conditions, C57Bl/6 mice which display the greater increase in septo-hippocampal cholinergic activation evidenced poor consolidation abilities (Galey et al., submitted for publication).

Additional analysis revealed in fact that these two parameters are negatively correlated. Thus it is suggested that the consolidation of informations would be possible only after a decrease of the level of cholinergic activation.

Finally, our data suggest the systems by which emotional states could act on informations processing and storage through the modulation of the septo-hippocampal cholinergic pathway.


The ECGs of CF and CA3 are rapidly and transiently expressed in response to intense smells in several species of experimental manipulation. Protein encoded by these rapid response genes may act as transcription factors that regulate the expression of downstream target genes, and so thereby function as mediators of long-term responses such as those necessary for the consolidation of memory. In support of this idea, Campeau et al. (1993) showed that conditioned fear significantly elevates a dorsal loci of amgual. We used c-fos and adf-26 per mRNA probes and in situ hybridization to investigate the effect of odor discrimination learning on the expression of ECGs in the rat brain. Initially we found that the learning rate for the first time and/or placing them in a novel environment (the training apparatus) eluates cis and trans-34 mRNA levels significantly above control levels in regions such as hippocampus and neocortical cortex. This expression was more pronounced in cis-34, and was greater in the CA1 than in the CA3 subfield of the hippocampal for both CEs. These differences were decreased significantly by repeated handling and intense in the training environment. To determine if specific brain regions are affected in experienced animals performing a novel odor discrimination, we compared cis and adf-26 expression levels in two groups of rats. Rats in both groups had been previously trained to solve seven odor discrimination between two simultaneously presented odors. Odor presentation was (5) 5'-19' performed on an eighth odor discrimination (for 30 min) and the remainder (e = 12) were simply placed in the training apparatus (for 10 and 30 min) as "control contexts." Animals were perfused 30 mins after their last experimental treatment. Animals performing the task had significantly higher c-fos levels in CA3 when compared to control contexts (p = 5%, barred runs). Such a change in expression was not found in the CA1 or any other region measured, nor was it found for adf-26 expression levels in the same region in adjacent tissue sections. These results suggest that selective areas of the brain elicit enhanced ECG induction/activation during odor discrimination behavior.

GENENDER DIFFERENCES IN BEHAVIORAL STRATEGIES AND RESPONSES TO CHOLINERGIC DRUGS. J.L. Sweitzer, B.L. Richardson and C.F. Holzmann, Wellesley College, Wellesley, MA; Johns Hopkins University, Baltimore, MD; Kennedy Krieger Institute, Baltimore, MD.
We have previously shown that basal forebrain lesions in neonatal mice lead to persistent behavioral impairments in adult mice. We now test whether cholinergic reconstitution therapy with physostigmine (PHY) can ameliorate the lesion-induced deficits. Our behavioral studies show both female and male mice become more impaired in performance in the swim maze task. Female gender is not readily detectable. Gender differences have been reported in other behavioral paradigms, the effect of lesion on performance in the control female and male population on a passive avoidance and spatial navigation task before and after administration of PHY. In passive avoidance, mice were injected with either saline or PHY (0.1 mg/kg, i.p.) after acquisition, then retention latencies were measured 24 hours later. In the spatial navigation task, mice were trained to swim to a hidden platform and then were injected with either saline or PHY (0.1 mg/kg, i.p.) 15-20 mins before testing. Small sex-related differences in performance existed in the absence of drug treatments, most notably, in acquisition of spatial navigation. Analyses of swim paths and patterns suggest that females and males use different strategies. The most drastic differences in performance, however, occurred in response to PHY. The same dose of PHY significantly increased passive avoidance latencies (7.6 ± 2.6 to 168 ± 12 s) and decreased swim latencies (19.9 ± 2 to 11.1 ± 3 s) in males but did not significantly alter performance in females. Full dose response curves are currently being generated. We characterize gender differences in response strategies and sensitivity to PHY and other cholinergic drugs.

LEARNING-ASSOCIATED CHANGES IN MONOAMINE RELEASE IN THE RAT BASAL FOREBRAIN. K. Tanaka., K. Horii., M. Oda., M. Iwaki and M. Nomura., Dept. of Pharmacol. Res. Lab., Toa & Co. Ltd., Chiba, Japan. We have previously shown that extracellular levels of monoamines and their metabolites were correlated with learning performance (Gallagher et al., 1987). To correlate extracellular levels of monoamines and their metabolites with learning performance, we have measured the monoamine and monamine metabolite content in the midbrain of rats (n = 12) which have acquired the operant brightness discrimination learning paradigm (Yamashita et al., 1987) during the first session. DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindole acetic acid (5-HIAA) levels during the learning test showed a trendancy to increase as compared to the pre-learning period. The percent increases in the monoamine and metabolite concentrations were then compared with various parameters of learning. We found a significant positive correlations between the percent increases in DOPAC, HVA and 5-HIAA concentrations and the correct response rate. The percent increases in the concentrations of metabolites, on the other hand, were significantly correlated with neither the total responses nor the reinforcements. These results imply that the monoaminergic inputs to the basal forebrain may play an important role in the learning process.

Recent work from this lab has shown substantial variation in the spatial learning abilities of aged rats, such that some aged rats are impaired in the Morris water maze performance, while other aged rats, as well as young rats. In the present study we assessed the effects of aging on nonspatial cognitive performance in odor-guided continuous delayed nonmatch to sample (cDNM). Rats were initially trained to perform the cDNM task under minimal delay and interference conditions, then challenged with longer delays and assessed for the effect of delay on performance. The same rats were then tested in the Morris water maze to compare performance across the two tasks. As in the maze task, we found a substantial variation in aged rats' ability to acquire the cDNM task. Aged rats were significantly retarded in acquisition of the cDNM task, however they were only slightly, if at all, impaired in later testing under increased delay-of-match methods. The cDNM tasks. These results reveal a relationship between the effects of aging on spatial and non-spatial tasks that may reflect a more broadly based deterioration in cognitive function than that commonly ascribed to a spatial memory impairment.
444.7 VIGILANCE AND SELECTIVE ATTENTION IN RATS USING AUDITORY STIMULUS DETECTION. P. J. Bushnell1 and K.L. Kelly2.
Vigilance involves maintaining attention to repeated stimuli over time, and selective attention may be inferred from changes in behavior due to manipulation of the likelihood (expectancy) of discriminative stimuli. To study vigilance, rats worked in operant chambers in which an auditory signal (a 20-msec increase of 1 to 7 dB in the intensity of continuous white noise) was presented on half of the trials. A food pellet was delivered if the rat pressed one of two retractable response levers on a signal trial, or if it pressed the other lever on a blank (no signal) trial. Signal detection analysis showed monotonic increases in signal sensitivity (sensitivity index, 5) and bias (response index, RI) with increasing signal intensity, showing that the accuracy of signal detection improved and the criterion for response to a signal became more stringent as signal intensity increased. Acute exposure to toluene vapor (2000 ppm) reduced SI at all signal levels greater than +1 dB without changing RI. Selective attention was studied with a similar procedure which required rats to detect either a 50 msec increase (+7 dB) or a 50 msec decrease (-6 dB) in continuous white noise. The probability of signal type (increase or decrease) did not affect SI when both signal types required the same response (e.g., press the left lever) and blank trials required the other response (e.g., press the right lever). However, the probability of signal type greatly affected response accuracy when each signal required a different response. These results suggest the importance of the response in demonstrating effects of expectancy on selective attention in rats.

444.9 MATCHING BEHAVIOR IN RATS SELF-STIMULATING IN THE MEDIAL FOREBRAIN BUNDLE. T. A. Mark1, J. C. Sim and C. R. Gallistel.
1Dept. of Psychol., UCLA, Los Angeles, CA 90024-1563.
Rats pressing two levers for brain stimulation reward on concurrent variable-interval schedules allocate their time between the levers in accord with relative reward abundance (subjective reward magnitude multiplied by rate of reward) - the matching law. This preparation may therefore provide a model system in which to study the neuropsychology of elementary computational processes. We studied the kinetics of matching after step changes in the relative reward magnitudes, the relative rates of reward, or both. Rats very rapidly altered their relative allocation (time on one lever/time on the other lever) in response to a change in either component of relative reward abundance. Transitions were commonly complete after one interreward interval on the leaner schedule. Relative time allocation tracked the random short term fluctuations in relative interreward intervals inherently present in two concurrent Poisson processes; hence, the extended to which run cannot be matched law depended on the method used to estimate overall reward rates and overall time allocations. [Supported by NSF Grant BNS 89-96266.]

444.10 REWARD SATURATION IN ELECTRICAL SELF-STIMULATION OF THE MFB. Janine M. Simmons2 and C.R. Gallistel.
2Interdepartmental Neuroscience Program, UCLA, Los Angeles, CA 90024-1563.
Rats press a lever to deliver a train of brief electrical pulses to the medial forebrain bundle (MFB). The time the animal spends on the lever depends on the remembered effect of the stimulus (the subjective magnitude of the reward). Increases in either current or pulse frequency increase the subjective reward magnitude, but only up to a frequency called the saturation frequency, above which reward magnitude no longer increases. We used a two-lever choice paradigm with variable interval schedules on both levers to determine the subjective reward magnitude as a function of pulse frequency at currents ranging from 1000 mA down to the 'current wall'. We find that the saturation frequency depends strongly on current, varying from 251-631 pps at the lowest currents (100-251 mA) to 63-200 pps at a 1000 mA. Increasing current shifts the function for reward-magnitude-versus-pulse-frequency to the left and sometimes increases its asymptote. These results make it unlikely that the saturation is due to a failure of frequency following in the first-stage axons.

444.11 EVIDENCE THAT HIPPOCAMPAL PLACE CELL REPRESENTATIONS OF MULTIPLE ENVIRONMENTS ARE NOT STRICTLY TOPOGRAPHIC. J.L. Kubie1, R.U. Muller1, B.M. Nawsey and C.P. Jia.
1Dept. of Anatomy and Physiology, S.U.N.Y. Brooklyn, Brooklyn, NY 11201.
The discovery of hippocampal place cells led to the hypothesis that the rat hippocampus is involved in spatial representations of the environment (O'Keefe and Nadel, 1976). Two questions that arise are: 1) How are multiple environments represented? and 2) Are the environments topographically mapped within the hippocampus? Previous work implied that there are independent representations of different environments (Muller and Kubie, 1987), but left open the question of topographic organization (Eichenbaum et al., 1990). In this study, we trained rats to chose food pellets in a square chamber and a cylinder. To date, we have recorded 7 sets of at least two cells in each environment. When two cells have firing fields in one environment, the relationship between the fields is called either overlapping or disjoint. We often see that a pair of cells with overlapping fields in one environment has disjoint fields in the second. These observations corroborate the idea of independent representations of different environments. They also strongly imply that the hippocampal representation of space is not topographic. In addition, we find that temporally correlated pairs of cells are less often recorded in the same environment. This result has consequences for theories of how the representation of a particular environment is established and maintained. [Supported by NIH NS20686.]

*Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563. Electrolytic lesions in the medial forebrain bundle at the junction between the posterior hypothalamus and ventral tegmental area were found to attenuate the rewarding efficacy of stimulation at the level of the lateral hypothalamus, as measured by the shift in the rate-frequency functions at several different current amplitudes. These results are consistent with findings by Waraczynski and her collaborators, that MFB lesions attenuate the rewarding efficacy of stimulation at sites rostral to the cut, but often have no effect on the rewarding efficacy of stimulation at sites caudal to the cut. A surprising finding was that our lesions had to be reasonably large (300 μA cathodal current for 200 s) before any decrease in rewarding efficacy of stimulation became evident.

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444.13 LESIONS OF THE NUCLEUS ACCUMBENS IN ADULT Rhesus Monkeys RESULT IN A DEFICIT OF MOTOR LEARNING BUT NOT IN S-R ASSOCIATIVE LEARNING OR MEMORY. R. Killiany* and H. Matul. Department of Psychology, Northeastern University, Boston, MA 02115

The results of the study demonstrated that lesions of the nucleus accumbens in adult rhesus monkeys resulted in a deficit of motor learning but did not affect associative learning or memory. This suggests that the nucleus accumbens plays a specific role in motor learning.

444.14 A METHOD FOR THE SEPARATION OF ATTENTION AND MEMORY IMPAIRMENTS FROM MOTIVATIONAL AND NON-SPECIFIC DEFICITS DUE TO WORK SCHEDULE SHIFTS. W.N. Tan†, B.L. Servatius, A.A. Petral, S.D. Drotar, M.T. Begic, V.A. Medical Center, E. Orange, NJ 07059 & New Jersey Med. Sch., Newark, NJ

This study presents a method for separating attention and memory impairments from motivational and non-specific deficits due to work schedule shifts. The method involves analyzing performance data to identify specific impairments.

444.15 BASAL FOREBRAIN (BF) LESIONS IN MONKEYS PRODUCE IMPAIRMENTS IN ATTENTION. M. Volytko*, M.S. Otten, R.P. Richardson and D.L. Price. Neuropsychology Laboratory, Johns Hopkins University Sch. of Medicine, Baltimore, MD 21205.

This study examined the effects of basal forebrain lesions in monkeys on attentional performance. The results indicated that basal forebrain lesions impair attentional processes.

444.16 TEMPORAL ORDER MEMORY IN MONKEYS IS IMPAIRED BY MEDIAL THALAMIC LESIONS. E.C. Gower* Boston VAAMC and Boston University School of Medicine, Boston, MA 02130.

This study investigated the effects of medial thalamic lesions on temporal order memory in monkeys. The results showed that medial thalamic lesions impair temporal order memory.

444.17 LOCUS COERULEUS UNIT ACTIVITY IN RESPONSE TO NOVELTY AND CHANGE ENCOUNTERED DURING ENVIRONMENTAL EXPLORATION. S.J. Sear, A. Vykroy and A. Beent. Inst. A. Beent, CNRS, GUYVETTE, FRANCE

This study examined the activity of the locus coeruleus in response to novelty and change encountered during environmental exploration. The results showed that locus coeruleus activity increased in response to new stimuli.


This study investigated the cognitive deficits in early-treated PKU (ET-PKU) due to dopamine depletion. The results showed that cognitive deficits were present in ET-PKU.

444.19 A NARROW BAND OF COGNITIVE FUNCTION IS POSSIBLY RESPONSIBLE FOR THE DECREASED PERFORMANCE ON SELECTIVE ATTENTION TASKS IN ADULTS WITH MODERATELY INTELLIGENT. B. Killiany, J. Komiyama, C. Lovett, M. Killiany. Department of Psychology, Northeastern University, Boston, MA 02115

This study examined the cognitive function responsible for decreased performance on selective attention tasks in adults with moderate intelligence. The results indicated that a narrow band of cognitive function is responsible.

444.20 IS THERE A HINT OF THE INHERITANCE OF COGNITIVE DEFICITS IN ADULTS WITH MODERATELY INTELLIGENT? B. Killiany, J. Komiyama, C. Lovett, M. Killiany. Department of Psychology, Northeastern University, Boston, MA 02115

This study investigated the inheritance of cognitive deficits in adults with moderate intelligence. The results suggested that cognitive deficits may have an inherited component.
445.1 LEARNING-INDUCED INCREASE OF TONE-EVOKED RESPONSE IN THE AUDITORY THALAMUS DURING PARADOXICAL SLEEP.
E. Havener*, C. Mabo and B. Hara. Laboratoire de Neurophysiologie de l’Apprentissage et de la Memoire, URA 1491, Université Paris XI, 91405 Orsay Cedex, FRANCE.
We have previously provided behavioral (Behavioral Brain Res., 18:241, 1985) and electrophysiological (Psychobiology, 19:193, 1991) evidences that a relevant stimulus can be detected during paradoxical sleep (PS). We report here that when an acoustic stimulus has acquired significance by conditioning during waking, its processing in the auditory system is enhanced during PS. After 1 session of habituation to a tone, waking rats underwent 3 conditioning sessions with the tone as CS preceding a footshock. After each session, the same tone was presented during PS periods, without any preceding of the animal. Multunit activity in the medial division of the medial geniculate body was recorded at each tone presentation during waking and during PS. After conditioning, tone-evoked response was increased both in the waking and PS states. No such changes were observed in pseudoconditioned animals receiving unpaired tone-shock presentations. Increased responses to meaningful stimuli, occurring in the sensory pathways on a background of limited responsiveness, could explain how an organism in PS is able to discriminate a relevant stimulus from all the other inputs.

445.3 INHIBITION OF DAY-TIME MEMORIES DURING NON-REM SLEEP AND SUPPRESSION OF MEMORIES OF DREAMS IN THE HIPPOCAMPUS.
Yoshinori Kuroda* and Satoshi Fujii*. Dept. of Molecular and Cellular Neurobiology, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo 183 and *Dept. of Biochemistry and Molecular Biology, Kyorin University, School of Medicine, Mitaka, Tokyo 181, Japan.
A "tracing circuit association" model has been proposed in which multimodal memory traces in cortical cell assemblies (tracing circuits) are consolidated by prolonged tracing through cortico-hippocampal projections and associated by LTP in intra-hippocampal connections (Kuroda, Y. Concepts in Neurosci.2:221, 1991). However, such multimodal associative memory connections in hippocampus could be easily saturated. Here, we propose a hypothesis that erasing of weaker connections occurs during non-REM sleep. Receptive low-frequency stimulation (reża=1 Hz) of excitatory input (depotentiation brought about by LTD) (310±50 mV in CA1 neurons in the slice, as well as long-term suppression of LTD (LTS; LTD cannot be formed for approx.60 min.). LTD is not induced by the activation (Pujol, S et al.: Brain Res.556:112, 1991). Such low frequency (1-2 Hz) activity of hippocampal neurons is observed as the slow wave during non-REM sleep in rat (Suzuki, S et al.: Electroenceph. Clin. Neurophysiol., 67:348,1987). Therefore, LTP of intrahippocampal connections can be reversed by DP with only strong LTD persisting after sleep. During sleep, especially REM-sleep, random associations of cortical activities (dreams) should be memorized by the association model, however, LTS caused by previous non-REM sleep may have inhibited formation of associative memory by LTP in REM-sleep. Some dreams may occasionally be scored by PTP and by strong LTD in the hippocampus which LTS fail to inhibit.

445.4 SUPPRESSION OF LTP INDUCTION IN THE DENTATE GYRUS DURING ALERT WAKEFULNESS BUT NOT DURING "ENHANCED" REM SLEEP AFTER AVOIDANCE LEARNING.
Rapid eye movement (REM) sleep is thought to play a critical part in memory formation. Learning events, such as active avoidance conditioning, are followed by a transient increase in the amount of REM sleep which is necessary for normal memory retention.

Rats were chronically implanted for unilateral stimulation of the perforant path and recording of evoked potentials in the dentate hilus. Test potentials were collected during REM sleep and a still-REM (SAL) behavioral state. After baseline recording rats were trained on a 40 trial shuttle-box avoidance task. Conditioned rats exhibited a significant increase in the duration of REM episodes relative to pseudoconditioned, yoked controls. High-frequency trains were applied during the second, third and fourth post-REM episodes. Another group was tetanized at the same time points during SAL. LTP was reliably induced during both SAL and REM following tetanization. In SAL, however, tetanization in SAL failed to elicit LTP of the EPSF slope (8% increase) while normal LTP developed after tetanization in "enhanced" REM (32% increase). There was no difference in the magnitude of population spike LTP.

We conclude that avoidance learning affects subsequent LTP induction in a state-dependent manner, allowing normal induction during post-REM REM but suppressing it during alert wakefulness.

445.5 SACCADES MODULATE UNIT ACTIVITY IN MEDIAL TEMPORAL LOBE IN THE DARK.
J. Ringo* and S. Sobota. Physiology, U. of Rochester
In primates, saccadic eye movements frame the visual input. They may then be triggered by an association of visual and mnemonic processing. Thus, we thought to look in the hippocampus and nearby temporal cortical areas for unit activity coupled to eye movements. Such responses turn out to be common, strong, and widespread, in both light and dark. It was found through 12 of 16 implanted guide tubes aimed at hippocampus, inferotemporal and medial ventral cortex.
In our sample analyzed to date (n>300, 3 monkeys), over 25% of the single units showed clear responses coupled to eye movements in the dark. For some cells, the altered activity begins 100ms before the saccade. Both increased and decreased activity were seen. Responses were usually dependent upon the eye movement's direction.
Post-saccadic time histogram analysis in the dark, triggered on each saccade (0 on abscissa). The ordinate is in spikes/sec.

445.6 A HEAD-FIXED PREPARATION FOR ELECTROPHYSIOLOGICAL STUDIES OF LEARNING AND MEMORY IN THE BEHAVING RAT.
A number of electrophysiological investigations of learning and memory could be more directly controlled and more easily accomplished in behaving rats if one could fix the animal's head in space and utilize recording techniques conventional in acutely anesthetized preparations. The successful development of a head-fixed behavior rat preparation depends on a long-lasting rigid head mounting system, techniques for adapting the rat to the fixed-head training environment, and selection of learning tasks in which such rats can perform well but experience brief rest periods. We have been developing such a preparation, adapting head-fixing and training techniques from recent successes with similar preparations by C.D. Woody and T. Ono, and exploiting rats' superb learning abilities when guided by olfactory cues.
Male Long-Evans rats were pre-trained on an odor discrimination task then, under ketamine-xylazine-anesthesia, implanted with multiple miniature self-tapping skull screws, a bipolar MFB electrode, and two horizontally-oriented cannulae on the skull surface. After recovery, they were habituated under successively smaller doses of acetaminophen to stereotactic fixation of the head using pins inserted into the calvaria. Then they were shaped to lick a water spout in order to receive sugar-water plus 400μA MFB stimulation. Later they were re-trained on the odor discrimination. Following these procedures rats could then be trained within a single session on novel odor discriminations. Furthermore, large single hippocampal complex spike and other cell types were readily isolated with brief pulses and could be recorded with stable, large signal-to-noise ratio during behavioral performance.

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The present study examined roles of hippocampal neurons by recording their single units while the rat performed auditory working and reference memory tasks. The working memory task was continuous nonmatching-to-sample (Sakurai, Behav. Neurosci., 104: 253, 1990). The reference memory task was continuous discrimination. The apparatus, stimuli, and sequence of the stimuli were identical for both the memory tasks. Around 10% of the units from CA1, CA3, and dentate gyrus showed behavioral correlates only in the working memory task. Behavioral correlates mean differences in unit's activity to the types of stimuli and/or responses in the tone presented and/or delay periods. Between 20% and 30% of the units showed behavioral correlates only in the reference memory task. About half of the units showed behavioral correlates both in the working and reference memory tasks. Types of these correlates were different between the tasks. These results suggest that there are different types of mnemonic neurons in the hippocampus and more of the neurons are related to both spatial and working and reference memory processes. (Supported by Grants-in-Aid Nos. 02552008, 02610040 and 03251213).

445.9 FUNCTIONAL SIGNIFICANCE OF ANATOMIC CONNECTIONS BETWEEN CAI AND CA3 HIPPOCAMPAL CELLS DURING DELAYED MATCH TO SAMPLE BEHAVIOR IN THE RAT

R.E. Hampson*, M.T. Kirby, K.E. Alexander, V.C. King and S.A. Deadpool. Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27157.

Recent studies of the intrinsic anatomic connections between the principal cells of the hippocampus (CA1 and CA3 pyramidal cells) have proposed an intimate gradient of connectivity which traverses the longitudinal axis of the hippocampus (Amaral & Winans, Neuroscience 31:771-991, 1989; Itohnsaka et al. J. Comp. Neurol. 295:580-623, 1990). The functional significance of these connections was investigated using many neuron recording and classification strategy to identify cells which appear to be functionally 'coupled' by these gradients in an in vitro monkey delayed match a delayed match to sample task (Heyser et al. Soc. Neurosci. Abstr. 15:170, 1989). Custom fabricated arrays consisting of 2 rows of 8 microelectrodes implanted to sample the longitudinal axis of the hippocampus, with the electrodes in each row positioned in either the CA3 or CA1 cells. Spike triggered histograms were constructed from spikes on each of the 8 microelectrodes for the entire set of spikes on the CA1 set 180 in 180 spikes in each of 5 10 s epochs. Extracellular spikes from individual cells were isolated by a multichannel online spike sorter (Spectrum Scientific) and cell firing correlated with various phases of the DMTS task. Several of the CA3 cells exhibiting task specific firing correlated also showed long latency 'coupled' discharges associated with CA1 cell spikes. Both the position and the degree of coupling between pairs of CA1 and CA3 cells across these electrode arrays as well as their relation to hippocampal activity will be described.

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445.11 BLOCKADE OF MITRAL/TCUTTED CELL HABITUATION TO ODORS BY ASSOCIATION WITH REWARD. D.A. Wilson* and R.M. Sullivan*, Psychol. Lab., Dept. Psychology, University of Oklahoma, Norman, OK 73019

Association of odor and reward during the early postnatal period modifies rat pup behavioral responses and olfactory bulb neural responses to subsequent presentations of that odor. Recent evidence from our lab has shown that olfactory bulb output neurons, including neurons in the mitral cell layer, receive convergent odor and reward inputs. Here, we examined the effect of reward input on M/T cell response to odors during associative training.

Single-unit recordings of M/T cells were made in PN11-12, urethane anesthetized Wistar rat pups. Responses of cells were examined during training, with odors presented and stimulation of the medial forebrain bundle/lateral hypothalamus. Odor responsive cells were then randomly assigned to either a PAIRED training group, receiving 4 sec odor pulses with MBFI/LH stimulation at odor offset or ODOR-only training. For both groups odors were delivered every 30 sec. Response magnitudes were measured in a control session (control LH stimulation) was compared across 15-30 conditioning trials.

The results suggest that contiguous odor-reward pairings prevent M/T cell habitation to the odor that normally occurs to repeated odor-only stimulation. Supported by NBS8819189 from NSF to DAW and DC05489 from NIH and BNS9110506 from NSF to RMS.

445.12 MODULATION OF THE EARLY AND LATE COMPONENTS OF EMG STARTLE ACTIVITY IN THE RAT

Laura L. Price and Valerie R. Nickerson, Psychology, St. Mary’s College of Maryland, St. Mary’s City, MD 20686

The reflex pathway of the acoustic startle response is a simple, fast-latency system within the brainstem of the rat. Short-term habituation of startle occurs within the reflex pathway, but long-term habituation relies upon the inhibition of the reflex response by neural mechanisms outside of the reflex circuit. The startle response also is modulated by other processes such as prepulse inhibition and potentiated startle. The short latency of the reflex places serious timing constraints upon these modulators of startle amplitude. The modulating effects of long-term habituation, prepulse inhibition, and potentiated startle on the startle reflex were studied using EMG recordings in the spinotrapezius muscle of 24 rats.

Acoustic startle stimuli provoked a bimodal EMG response with response latencies of about 8 and 18 ms. Preliminary evidence suggests that the late component may undergo stronger long-term habituation than does the early component. Both components, however, respond to changes within a test session, including manipulations of stimulus intensity and interstimulus interval. Furthermore, the amplitude of both components decreased during prepulse inhibition testing. Preliminary evidence suggests that the responses of the two EMG components are not altered within a potentiated startle paradigm when the startle stimulus serves as the conditioned stimulus paired with aversive footshock.
445.11

EFFECTS OF DORSAL CORTEX LESIONS ON HABITUATION TO A LOOMING STIMULUS IN TURTLES. A. S. Footner* and L. Mylick.
St. John's University, Jamaica, NY 11439.

Previous investigations in our laboratory have shown that dorsal cortex lesions impairing habituation. The present study was undertaken to determine whether habituation to a looming stimulus (LS) would be affected by such lesions. Painted turtles (Chelydra serpentina) were given dorsal cortex (n=4) or sham lesions (n=5). The apparatus consisted of a platform on which the turtle was restrained in front of a rear-projection screen on which the LS was presented. Head withdrawals were detected by a photocell beam that was shadowed when the subject's head was extruded. Turtles with sham lesions showed rapid habituation to the restraint and showed both within- and between-day habituation to the LS. Turtles with dorsal cortex lesions took significantly longer to habituate to the restraint, and 2 animals failed to show such habituation. There was a nonsignificant suggestion of an impairment in between-day habituation in the 2 turtles that were able to be tested on habituation to a LS. The results suggest that dorsal cortex lesions may produce a deficit in habituation in turtles.

445.14

A FREQUENCY ANALYSIS OF HABITUATION IN STATIONARY RESPONSE-VISUAL CELLS IN THE AMPHIBIAN TECTUM. M. M. Nikoletses.* Dept. of Anatomy, U. of Puerto Rico, Sch. of Med. San Juan, PR 00936.

Tectal cells of various sensory modalities were reported to be habituated. Habituation is traditionally defined as a decrement in the number of responses as a function of repeated stimulus presentation. In this study, a quantitative analysis of habituation was undertaken with emphasis on the changes in interspike intervals. Frogs (Rana catesbeiana) were anesthetized and paralyzed. A light stimulus (duration 0.50 ms, ISI: 100 ms) was presented in the center of the receptive field of stationary response-cells (SR-cells) (movement-sensitive and directionally-selective cells were excluded) for 1.5 min, and spikes were preamplified and fed into a computer for off-line acquisition and analysis. Use of various filters excluded stimulus artifacts and noise from the analysis. The main findings are: 1. These cells had very low spontaneous activity. 2. The increment in response rate as a result of stimulus presentation gradually declined with repetition but never reached spontaneous levels. 3. As habituation progressed interspike interval lengthened (coding by frequency). 4. In spontaneous recovery there was generally a return to high response rates and short interspike intervals. 5. The frequency coding mechanism is independent of that of spike count. In conclusion, during habituation SR tectal visual cells employ the same coding mechanisms as do somatosensory and auditory tectal cells previously reported.

446.1

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In a recent selection study we have reported that rats fed dietary tallow consume increased amounts of high protein/low carbohydrate diet compared to control, corn oil fed rats (J. Nutr. 120: 1418, 1990). These studies suggest that some factor or condition associated with ingestion rates influenced rats diet in a shift in diet selection. In this study we examined the effect of AP/mNTS-lesions on selection of a high protein/low carbohydrate diet versus a low protein/high carbohydrate diet. We found that rats that received an AP/mNTS-lesion at least 12 weeks earlier selected a greater percentage of their daily diet from the high protein/low carbohydrate diet than unlesioned control rats (AP/mNTS-lesion, 41.5 ± 10.4% vs. CON, 15.1 ± 3.7%). Weight loss by the lesioned group does not appear to explain altered diet selection as weight matched control rats consumed only 2.24% of daily intake from the high protein diet. These data provide support for a role of the AP in diet selection. (Funded by NIH DK42533)

446.2

LESIONS OF AREA POSTREMA (AP) ABOLISH CONDITIONED TASTE AVERSIONS BUT NOT AOREXIA INDUCED BY LICI IN RATS. K. S. Curtis, A.P. Ryed, J.G. Verbalis, E.M. Stickert., Dept. of Behavioral Neurosciences, University of Pittsburgh, Pittsburgh, PA 15260.

AP lesions were produced by vacuum aspiration in adult male Sprague-Dawley rats. When given with previous findings, when water-deprived rats were allowed to drink flavored fluids just before treatment with LICI (3 mg/kg, ip), sham-operated (n=8) and non-operated control rats (n=7) demonstrated a pronounced aversion to the fluids whereas rats with AP lesions (n=12) did not decrease fluid consumption significantly. However, in a 30-min test period after overnight food deprivation, rats with AP lesions and control animals reduced food intake significantly and to an equivalent degree after pretreatment with either LICI (3 mg/kg, ip) or hypertonic saline solution (2-3 ml of 2 M NaCl, ip). These results are consistent with the traditional view that AP mediates the sensation of nausea produced by LICI treatment (hence the loss of conditioned taste aversions after AP lesioning) but suggest that neither nausea nor AP is critical for the marked disinclination to eat that is induced in rats by systemic treatment with LICI or with hypertonic NaCl solution.

446.3

Neuroscience Program, Univ. of Western Ontario, London, Ontario, CANADA N6A 5C2.

The present study examined the role of the area postrema (AP) in mediating the effects of lithium on taste reactivity (TR) responses. Rats received lesions of the AP (APX, N=12), or sham lesions (APS, N=14), followed by implantation of an introral cannula. On the first test day, half of the rats in each group were injected with either LiCl or equimolar NaCl (0.6 M, 5 ml/kg, i.p.). Ingestive TR responses (rhythmic mouth movements, tongue protrusions, and lateral tongue protrusions) and aversive TR responses (vomiting and chin rubs) were videotaped during 30 sec intraoral infusion of 0.3 M sucrose. Infusions began immediately post-infusion and at 5 min intervals for 30 mins. Three days later this procedure was repeated. Rats were tested 72 hours later for the expression of a conditioned taste aversion. APS rats given NaCl showed higher levels of ingestive responses during all trials. APS rats given LiCl showed a decline in ingestive responses and an increase in aversive responses (p<.001). In comparison, APS rats treated with either LiCl or NaCl displayed high levels of ingestive responses similar to the APS/NaCl group (p>.10). It was concluded that lithium-induced activation of the AP is required to produce palatability shifts and the formation of CTAs. (Supported by a NSERC operating grant to KPO).

446.4


Increased extracellular ACh in the nucleus accumbens (NAC) is associated with precipitated morphine withdrawal (Rada et al. 1991) and suppression of feeding (Mark et al., 1991). If these findings indicate an aversive action of the transmitter, then ACh increase in the NAC may be sufficient to induce a conditioned taste aversion.

Male Sprague Dawley rats were water deprived and trained to drink during 30 min. daily access to water. After training, animals were presented 2.5 mM sodium saccharin in water, and the taste was paired with either 4 µg/µl neostigmine (n=7) or vehicle (n=7) injected bilaterally in the NAC (0.5 µl per side). A pseudoconditioning control group received NAC in the NAC paired with water presentation (n=7). In a two bottle preference test, the saccharin-neostigmine group showed a significant aversion to saccharin (P<2.18) x15.57, p<.0001) compared to the two control groups. There were no differences in overall fluid consumption. These results suggest that an increase in extracellular ACh in the NAC may be sufficient to produce an internally aversive state.

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Male rats were chemically laryngectomized by intratracheal injections of sodium carboxylate (Gyn VNS). Control rats were given intratracheal injections of isotonic saline (Group VNS). All rats were exposed to a 23 hr/day water deprivation schedule and then exposed either to a conditioned taste aversion (CTA) procedure or a control procedure. The CTA technique involved the pairing of a novel saccharin taste (0.15% solution) with subsequent i.p. injection of ethanol (1.5 g/kg, 15% solution). The control procedure involved the pairing of the saccharin taste with an injection of isotonic saline (10 ml/kg). Following 2 conditioning trials and 3 days of water only, saccharin preference ratios were obtained (2-bottle choice test) on 4 consecutive days. VNS rats exposed to saccharin plus ethanol exhibited a strong CTA (p<0.01) relative to VNS controls. VNX rats given saccharin plus ethanol showed a strong CTA (p<0.01) if conditioning occurred 29-30 days post laryngectomy. However, CTAs were abolished in VNX rats conditioned 19 days post laryngectomy. Thus, ethanol-induced CTA formation varied across the post laryngectomy time period. (Supported by a NSERC operating grant to KPO).

466.7 EVIDENCE OF LEARNING IN THE ETIOLOGY OF POISON-ELICITED PICA. D. Mitchell* and J. B. Nast. Department of Psychology and Psychobiology Program, University of Southern California, Los Angeles, CA 90089-1061.

Both humans and other animals frequently engage in pica (the consumption of non-nutritive substances) when suffering from gastrointestinal malaise. We have previously shown that a specific form of pica, geophagia (clay consumption), can be used to quantify the severity of gastrointestinal malaise induced by a variety of toxins and emetics, including lithium chloride. Though a small dose of lithium chloride reliably elicits robust conditioned taste aversions, the same dose elicits only a modest pica response compared to other toxins and emetics. This apparent inconsistency between the robust effectiveness of lithium chloride as a UCS in conditioned taste aversions and its modest effectiveness in eliciting pica led us to examine the putative role of learning in the etiology of poison-elicited pica.

Rats maintained with food, water, and kaolin always available were administered repeated intraperitoneal injections of 1.5 M lithium chloride (127.2 mg/kg) every fifth day for a total of ten trials. Results showed that the animals learned to engage in pica; clay consumption gradually increased across the first five trials from 1.5 g on the first trial to 15.3 g on the fifth trial. Thereafter, it remained stable across the remaining trials. These results are consistent with clinical literature which suggests that learning plays an important role in the etiology of pica.


Following adrenalecetomy, rats consume less food than sham-operated controls. If adrenalecetomy produces illness or nausea, it is possible that adrenalecetomy (ADX) rats show reduced food intake because they have developed a conditioned aversion to the diet consumed following surgery. To test this hypothesis, rats were introduced to a novel diet (either C-21 or AIN) for a period of seven days immediately following adrenalecetomy or sham-adrenalecetomy. ADX rats consumed significantly less food than sham-ADX rats during this period. On the eighth day following surgery, a preference test was administered. Rats were 8 hr. food-deprived, and then provided 4 hr. access to both C-21 and AIN. Mean 4 hr. food intakes for each of the groups, expressed as grams of C-21 consumed divided by total consumption during the preference test, were: sham C-21-maintained, 86; ADX C-21-maintained, 95; sham AIN-maintained, 34; ADX AIN-maintained, 35. Statistical analysis by ANOVA revealed that ADX groups did not show significantly lower preference than sham ADX groups for the diet to which they were exposed during the week following adrenalecetomy. ADX rats tended to consume a higher percentage of their food as C-21 than sham-ADX controls during the preference test, but this trend was not significant. These data do not support the hypothesis that decreased food intake following adrenalecetomy is due to illness or malaise.

466.9 WHERE IS AVERSION: LATERAL HYPOTHALAMUS OR VENTRAL PALLIDUM/SUBSTANTIA INNOMINATA? H.C. Cromwell*, D. Baraban and C. Unger. The University of Michigan, Department of Psychology, Ann Arbor, MI 48109

Many previous studies have reported that LH damage induces aversion (Tolka et al., 1976; Bliss et al., 1978). However, a study of neuron loss restricted to the LH found aphagia without aversion (Berridge, 1989). The purpose of this study was to resolve where the crucial site for aversion inducing lesions was located. Small bilateral excitotoxic lesions (QUIN, 60nm in 0.5ml of PBS) or bilateral sham injections of the lateral hypothalamus (LH), nucleus accumbens (NAcc), or the ventral pallidum (substantia innominata, VP; SI) were completed to determine the exact locus related to aversion enhancement. The taste reactivity test (Gill and Norgren, 1978) using oral taste infusions of sucrose (1M) was completed and the number of aversive responses (gasps, chin-rubbing, head shaking and forelimb flail) to the palatable sucrose were tallied. To identify the lesions, two lesion mapping techniques were used: 1) a conventional neuron counting procedure, in which an attempt is made to count all neurons within a brain region, and 2) a new modified 'fractionator' procedure consisting of accurate 40X magnification counts at many point locations within a brain region. Results indicated that food aversion is produced following bilateral neuron loss exceeding 70% in a 500ym diameter area of the caudal ventromedial VP/SI alone, and not from NAcc or anterior LH damage. The crucial adrenergic targets are medial to the globus pallidus, and dorsal and lateral to the lateral hypothalamus.

466.10 2,5-Anhydro-D-mannitol (2,5-AM) INCREASES FOS-LIKE IMMUNOREACTIVITY (Fos-Li) IN THE AP InVERSE REGION BY STIMULATING VAGAL SENSORY NEURONS. T.T. Drescher, B. Ritz and M.L. Friedman, Washington State University, Pullman, WA 99164-6520 and Monell Chemical Senses Center, Philadelphia, PA, 19104.

The antinociceptive effects of 2,5-AM, 2,5-hydroxybenzylamine, and 2,5-dimethyl-2,5-dihydroxybenzylamine (2,5-DA) were examined in rats to determine whether the effects were centrally mediated or not. Rats were intraperitoneally injected with 1.0, 2.5, or 3.0 mg of 2,5-AM. At each dose level, 2,5-DA was also administered. The results showed that both compounds produced significant decreases in pain-related behaviors, but not all effects were centrally mediated. The effects of both compounds were blocked by a centrally acting opioid antagonist, naloxone, but not by a peripheral antagonist, 2,5-DAM. These data suggest that 2,5-AM may act on both central and peripheral sites to produce its effects on pain behavior.
446.11 CHARACTERIZATION OF GASTRIC VAGAL AFFERENT MECHANOSENSOR RECEPTORS TO CLOSE ARTERIAL INFUSIONS OF CCK IN RATS: G.J. Schwartz, P.P. McHugh, & T.H. Moran. Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We have shown that gastric vagal mechanoreceptors also respond to close arterial infusions of the brain-gut peptide CCK. To examine the pharmacological specificity of these CCK-elicited responses, we compared the chemosensory activity of single rat cervical vagal afferent fibers sensitive to both gastric loads and CCK (N=14) before and after administration of the selective A and type B CCK receptor antagonists, L-364,718 and L-365,260. Some of these fibers (N=9) were also monitored for their responses to the above stimuli after acute pylorotomy. In addition, we investigated the ability of JMV-180, a selective CCK-1 receptor antagonist, to attenuate the responses to CCK, both before and after acute pylorotomy, without altering the response to gastric loads. L-365,260 (10 pmol/ml) failed to attenuate the gastric vagal afferent response to either gastric saline loads or 100 pmol infusions of CCK. In intact rats, celiac artery administration of 100 nmol of JMV-180 completely blocked the ability of 100 pmol CCK to stimulate gastric afferent fibers, again without attenuating the response to gastric saline loads. These results demonstrate that CCK-elicited responses in a population of gastric vagal mechanoreceptive fibers are mediated by non-pyliotic, type A CCK binding sites similar to pancreatic low affinity CCK receptors. Supported by DK19302.


Following bilateral removal of the superior cervical ganglion, pseudobacteria (vir 15 ul, 8x10^5 pfu/ml) was injected into the anterior digastric muscle, or temporal muscles of rats. The animals were allowed to survive from 72 to 120 hours. Primary injections were restricted to trigeminal motor neurons (Mo 5) in a predictable myotopic pattern. Secondary injections appeared in neurons in areas known to project to Mo 5, including the Kollik-Fuse, parabralachial, and principal trigeminal nuclei, as well as the supratrigeminal and subcoeruleus zones. Within these areas, however, infections of different muscles produced different distributions of labeled cells. Similarly, differential labeling was observed in regions thought to be involved in the central pattern modulation of Mo 5 activity, such as the gigantocellular and paragigantocellular areas. In the brainstem, increasing survival times revealed polysynaptic connections to the subpedunculopontine and pedunculopontine nuclei in the midbrain, the lateral tegmental and the oral, ventral, and caudal pontine nuclei in the pons, and the parapontalgial, solitary, and rostroventromedial nucleus in the medulla. With longer survival times the infection also spread to the forebrain, particularly in the hypothalamus, amygdala, and olfactory lobes. Nevertheless, the neocortex, thalamus, and most of the striatum remained remarkably free of labeled neurons. Supported by PHS grants DC 00240 and MH 00653.


Lesions of the area postrema/nucleus of the solitary tract (API/NTS) abolish lipoprotein and glucoprotein feeding induced by metoclopramide (3 mg/kg and 2-DOG) respectively. Since the IPBN is a major relay site for ascending visceral sensory input from the AP/NTS region, electrolytic and ibotenate lesions of IPBN subnuclei were made to investigate their involvement in the central pathways of metabolic control of food intake. Lesions were confirmed either by cresyl violet staining or by glial fibrillary acidic protein immunohistochemistry. Lesioned rats were tested for feeding in response to 0.2% NaCl (s.c. or i.p.), MA (400, 800, and 800mg/kg, i.p.) and 2DG (100 and 200 mg/kg, s.c.). Electrolytic lesions of the dorsal, central, superior and external IPBN abolished lipoprotein but not glucoprotein feeding. Ibotenate lesions of the external and superior subnucleus did not impair lipoprotein or glucoprotein feeding, but ibotenate lesions of the dorsal and central subnuclei did abolish lipoprotein feeding. These results suggest that the cells bodies in the dorsal or central IPBN control lipoprotein and glucoprotein feeding. Electrolytic lesions of the inferior and superficial subnuclei appear to have damaged fibers of passage important for this control, possibly those in transit to or from the dorsal and central subnuclei.

446.16 REFEEDING AFTER 48 HOUR FAST INDUCES C-FOS mRNA AND PROTEIN IN RAT BRAINSTEM AND HYPOTHALAMUS: T.A. Hoerr, T.C. Wessel, T.H. Ishi, and A.C. Towle. The Bourne Lab. and the Burke Medical Research Institute, Cornell University Medical College, New York, NY 10032.

In order to identify specific brain regions stimulated at the transcriptional level by food ingestion, we examined the expression of the proto-oncogene c-fos in brainstems and hypothalami by immunochemistry and in situ hybridization in three groups of rats: ad lib. fed controls, rats fasted for 48 hrs, and rats fasted for 48 hrs and allowed to eat Purina Lab Chow ad lib. (50 animals per group). For in situ analysis, 4um tissue sections were hybridized overnight with a [35S]-labeled c-fos oligonucleotide. Sections were dipped in emulsion and developed 4-6 days later. Adjacent sections were immunohistochemically stained for c-Fos-related proteins.

One hour of feeding following a 48 hr fast caused intense c-fos mRNA expression and immunoreactivity to be localized in several regions of the brainstem. The expression was very strong in the nuclei of the solitary tract; in the rostral hypothalamus, strong c-fos expression was seen in the paraventricular and supraoptic nuclei, and more diffuse expression was seen in the anterior hypothalamic nuclei and bed nuclei of the stria terminals; at the level of the caudal hypothalamus, the dorsal medial nuclei and the thalamic habenular and paraventricular nuclei were labeled, as well as individual cells encircling the third ventricle. Control and fasted animals showed little or no expression compared to refeled animals in brain stem and anterior hypothalamus, although ad lib. fed controls showed occasional c-fos expression around the dorsal medial nuclei in the caudal hypothalamus.

Our results show that feeding induces c-fos expression in specific brain nuclei at both the mRNA and protein level. Further studies are required to correlate c-fos expression in these neural areas to specific homeostatic and postingestive effects of food intake.

446.17 MEDIULLARY INPUTS TO THE FIFTH, SEVENTH, AND TWELFTH CRANIAL NERVE MOTOR NEURONS: H.T. Cunningham, J.H. and F.L. Sawchenko. The Sak Institute, Los Alamos, NM 87545.

Prior anatomical studies performed in the rat identified the region of the caudal medullary reticular formation immediately subjacent to the nucleus of the solitary tract (NTS) as the primary source of afferent inputs to the motor nuclei of the fifth (MoV), seventh (VII), and twelfth (XII) cranial nerves, which are involved directly in the control of oromotor behaviors such as deglutition and mastication. In this study, the sensitive fluorescent retrograde tracing technique was used to identify medullary areas innervating MoV, VII and XII, and to characterize the cells of origin of these projections. Discrete deposits of the anterograde tracer Fluorogold, Fluororo-dioxygenase, were then placed in implicated portions of the dorsomedial reticular formation, and the topography of labeled terminal fields within each of the cranial nerve motor nuclei were charted. The results may be summarized as follows:

1) The regions of the reticular formation innervating MoV, VII and XII are largely overlapping, and extend from the level of the MoV to the dorsal spinal cord. In each instance, the greatest concentration of retrogradely labeled cells lay at the level of the NTS.
2) Most inputs are bilateral with an ipsilateral predominance. The single exception is the largely contralateral input to motor neurons innervating the anterior digastic and mylohyoid muscles in the ventromedial portion of MoV. They also project to the intermediate and dorsal portions of VII, and to all subdivisions of XII regions that innervate a number of muscles involved in the initiation of deglutition. Thus, although the resolution of these techniques does not allow us to discount the possibility that a small proportion of neurons in the reticular formation might share characteristics of both populations, it appears clear that two distinct, though topographically contiguous, deglutitive and masticatory pathways arise from the reticular formation in the dorsomedial medulla.


Duodenal lipid infusions rapidly suppress sham intake of liquid diets and rapid stimuli. These effects were observed following sham gastric equipotent T.c. and duodenal cannula, maintained on a food deprivation schedule, and trained to ingest fluids while restrained in a chronic recording apparatus. Parabralachial gustatory neurons were identified using conventional recording techniques. These cells were tested with intralipid infusions (50 ul) of a concentration series of sucrose, as well as a single concentration of policy of the ileum, citric acid, and quinine HC1 before, during, and after duodenal infusions of a lipid emulsion (Intralipid) or saline. The neurons were tested during 3 different, 15-minute periods, before and after the duodenal infusions. The responsiveness of at least 7 taste neurons was assessed during each time period; 11 neurons were tested both before and after an infusion. During the first post-infusion interval (0-15 min), neural activity elicited by NaCl decreased to less than that produced by any concentration of sucrose. During the next time period (15-30 min), taste receptors responded to lower concentrations of sucrose, but increased their responsiveness to NaCl. During the last period (45-60 min), NaCl responses returned to pre-infusion levels, while sucrose responses increased. These data suggest that some neurons may switch their best response category depending on whether the animal is food deprived or satiated. Supported by DC-00047, DC-00240, MH-00653.

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CELLS WITHIN PONTINE PARABRACHIAL NUCLEUS COLLABORATE TO INHIBIT ACTIVATION OF PAGMUS AND OVERARCHING MEDELINA IN THE RAT: IMPLICATIONS FOR FUNCTIONAL INTEGRATION OF PAIN AND SATIETY. L. Belayance, P. L. Fanta, R. D. Hofbauer, and C. Gonzalez. Division of Neuroscience Research, University of Minnesota, Minneapolis, MN 55455.

The pontine parabrachial nucleus (PNB) receives input relevant to feeding and nociception, and may play an integral role in the synchronization of these different functional systems. In this part of the study, we utilized the minigrade tracers to label lateral and/or adjacent to the pontine parabrachial nucleus (PNB) and to determine if a single cell in the PNB collateralizes to innervate two distinct brain areas, thus increasing information relevant to feeding or nociception. After injection of rhodamine-conjugated latex beads into the dorsal medulla and injection of fluorescently into the hypothalamus, doubled labeled cells were located in the pontine parabrachial complex. This would suggest that some PNB cells collateralize to innervate both the dorsal medulla and the hypothalamus, structures involved in the regulation of feeding and nociception. This second part of the study was designed to look at the peptide content of the collateralizing PNB cells. We combined the retrograde fluorescing immunofluorescence technique to determine whether the PNB cells that collateralize also co-localize peptides implicated in the transmission of information relevant to feeding and/or nociception: cholecystokinin (CCK), neuropeptide Y (NPY), enkephalins. The results of these studies suggest that the PNB may utilize collateralization as an integrating system in addition to the more traditional interneuronal activities. Furthermore, the PNB may play an integral role in the synchronization of different functional systems, i.e. feeding and nociception.


Hyperphagia and obesity in cats, dogs, and primates with lateral amygdaloid lesions were observed by several investigators during the 1950's, 1960's, and early 1970's (e.g., Bunberg, 1955; Forsberg, 1971; Greenfield, E. 1997) and Clemente & de Groot, 1957). Interest in this extra-hypothalamic obesity syndrome disappeared when such effects were not observed after amygdaloid lesions in rats. In an initial part of the present study, we also found that large lesions of the amygdala (ylisis had not been completed at the time of submission) resulted in weight gains of 15-25 grams during the first 2-4 postoperative days. Daily food intake nearly doubled in some animals. In a subsequent controlled study, adult female rats with amygdaloid lesions gained 20-30% of their preoperative body weight in 30 days, compared to only 5% for animals with sham lesions. The amygdaloid-lesioned animals were unaffected when switched from a standard pellet diet to a high-fat diet and back again. It is hypothesized that the amygdala plays a major role in appetitive mechanisms.


Neuroanatomical studies have implicated the reticular formation (RF) lateral to the hypoglossal nucleus (xII) as a substrate interposed between brainstem orosensory and motor nuclei. This substrate may be important for producing ingection (licking and swallowing) or rejection (gaping) responses to gustatory stimulation. In the present study, rats were implanted with a chronic microdevice that advanced a bundle of fine wires into the RF lateral to the hypoglossal nucleus. A total of 80 single cells (8 multi-unit responses) were recorded from 18 preparations. Thirteen preparations had recording tracks through the center of the solitary tract (NST) and the RF lateral to xII from a level 0.2 mm rostral to obex to the rostral pole of xII. Electrode tracks ranged from the lateral border of xII to 0.5 mm lateral to the nucleus. Five preparations had electrode tracks extending from the rostral pole of xII to a level 2.5 mm rostral to obex and from 0.4 - 1.0 mm lateral to the midline. Oro-nasal responses to intraoral fluid stimulation were recorded from all preparations (43/80 cells) except two, the medial or the lateral larynx. Responses specific to swallowing were recorded dorsal to oro-lymphatic structures in 5 preparations and were located in the NST at levels ranging from 0.4 - 1.4 mm rostral to obex. Thus it would appear that the caudal NST and the RF ventral to it (lateral to xII) mediate different aspects of the ingestive sequence. Supported by DC04147.
447.1

**D. DOPAMINE RECEPTOR ACTIVATION INDUCES SOCIAL REACTIVITY IN MICE SELECTIVELY BREED FOR AGGRESSION.** M.H. Leskic, L.L. Garvey, P. Gendreau, M.A. Mayleden, D.E. Nichols, R.B. Mulimani, B.H. and Development Research Center, 11th, Dept. of Psychology, Psychology and University of North Carolina, Chapel Hill, N.C., 27599 and School of Pharmacy, Purdue University, West Lafayette, IN, 47907.

Robust social behavior has been examined in mice bred for selective breeding ICRC mice for high and low levels of aggression. As previously shown, when paired with a non-selected, group-housed partner mouse, NC900 mice exhibit increased levels of aggression, whereas NC100 mice fail to attack, but rather freeze upon social contact. Previous studies have established that NC100 mice have lower dopamine concentrations in nucleus accumbrans and caudate nucleus, with increased dopamine receptor density in these brain regions. Thus, we wished to determine the effect of administration of a dopamine receptor agonist on social behavior. Mice of both lines were administered 0, 1, 3, or 10 mg/kg (s.c.) of the agonistic, full efficacy, dopamine receptor agonist dDAVP and their behavior assessed in a social interaction test. Dihydrexidine dose-dependent reduced aggression in NC900 mice. Instead of aggression, these animals displayed a marked reactivity to mild social stimuli, as measured by increases in escape behavior, reflexive kicks, and vocalizations. Dihydrexidine had no systematic effect on the freezing behavior characteristic of the low-aggressive line, but did induce social reactivity in these animals, albeit to a lesser degree. In independent experiments, mice were pretreated with either the D2 agonist SC123190 (0.1 mg/kg) or the selective D2 agonist remoxipride (1.0 mg/kg), after which they received dihydrexidine (10 mg/kg) and were tested as above. The effect of dihydrexidine on social reactivity (both NC900 and NC100) and attack (NC900) were significantly antagonized by SC123190 but not attenuated by remoxipride. These studies suggest an important role for D2 dopamine receptors in mediating social stimuli (Supported, in part, by PHS Grants MH45371, MH54587, and MH42765).

447.3

**INTRACRANIAL SELF-STIMULATION ON FR15 PRODUCES A BEHAVIORALLY SENSITIZED RESPONSE TO LOW DOSES OF COCAINE HYDROCHLORIDE (COC) IN THE RAT.** E.R. Hartley* and D.E. Reilly.

Department of Psychology, Emory University, Atlanta, GA 30322.

In a previous study (Neurosci Abst, 1990) we report that rats performing ICSS at L8 electrodes on an FR15 schedule showed a complete cessation of responding when administered low-dose AMPH unlike animals trained on an FR1 schedule. The FR15 rats developed increasing levels of stereotypic behavior over the course of the 20 minute session and a dose-response analysis revealed a left shift in FR15 as compared to FR1 rats. To determine if the above behavioral augmentation occurred outside the operant environment, rats were assessed in their home-cage environments. No differences in stereotypy ratings between groups were observed.

In a separate study, the cocaine Coc was examined. A similar response suppression was observed in rats performing on the FR15 schedule though the stereotypy that was observed was qualitatively different as it was statically different to the AMPH-induced stereotypy. Dose-response analysis of Coc revealed a left shift in FR15 rats but, unlike AMPH, there was some response suppression at all doses. A home cage assessment between groups revealed no differences in the stereotypic response to COC and measures of locomotor behavior obtained immediately following ICSS were also no different.

We propose that the FR15 schedule induces an elevated DA release only within the context of the operant environment and that the addition of the AMPH or COC results in an elevated baseline resulting in the development of stereotypic behaviors that compete with the performance of the lever press response.

447.5

**EFFECTS OF DOPAMINE BLOCKADE ON THE HYPERACTIVITY PRODUCED BY DRUG INJECTIONS INTO THE MEDIAN HIPPOCAMPUS.** L.S. Kiro and C. Mcshaffer, Dept. Psychol., Univ. Ill at Chicago, Box 4348, Chicago, I1 60680.

We have shown previously that injections of GABA and opioid agonists into the median raphe nucleus (MR) result in large increases in both locomotor activity and dopamine turnover within the nucleus accumbens. In experiments conducted, we examined the possibility that the MR's influence on locomotion might be mediated by alterations in dopamine release. Hyperactivity was induced by injections of intrar- MR injections of DAGO or DIPPE. In contrast, haloperidol, at a dose of 0.4 mg/kg, was able to abolish the hyperactivity induced by systemic amphetamine or by intra-MR injections of DAGO or DIPPE. In haloperidol, hyperactivity was without effect on the response to intra-MR injections of muscimol or baclofen. These results suggest that dopamine may be involved in the response to intra-MR injections of intrar- MR, but is unlikely to play a critical role in the response to GABA agonists. These data also suggest that different populations cells may mediate the locomotor responses to intra-MR injections of GABA and opioid agonists.
447.7 DIABETIC RATS DISPLAY BLURRED BEHAVIORAL RESPONSE TO D-AMPHETAMINE OR DARK PHASE; IMPLICATIONS OF ALTERED DOPAMINE (DA) FUNCTION. Q. AHMAD AND Z. MERALI* Sch. of Physiol. & Dept of Pharm. Univ. of Ottawa, Ont. Canada, K1N 8B9.

The behavioral response of spontaneously diabetic Wistar BB rats (SDR) (maintained on insulin) and matched controls (CTL) to systemically administered d-amphetamine (d-AMPH) was compared. Diabetic rats (4-6 months) and long-term (8-12 months) stages of diabetes, was assessed. Each stage comprised of a different set of animals. Behaviors monitored for 1 h per day included locomotion, general activity, rearing frequency and duration. During the early stage, d-AMPH (0.3, 0.5, 10 mg/kg) induced a dose dependent increase in behavior of CTL rats. The SDR group only responded to the higher doses (0.5 and 1.0 mg/kg of d-AMPH and with a smaller magnitude than the CTL group. At the intermediate and long-term stages, the SDR again displayed a shift to the right of the d-AMPH dose response curve. Similarly blunted behavioral activity was also noted in the SDR during the dark phase of the light cycle. Specifically, the SDR displayed lower levels of activity at light offset or before light onset. Thus the altered response of the SDR may be related to the impairment of synthesis and/or release of DA. This contention was tested in SDR (intermediate state), using microdialysis. Extracellular levels of DA at the nucleus accumbens were monitored every 20 min during the dark phase, in behavior animals. The SDR had a higher basal level of DA as compared to CTL. However, the SDR displayed blunted fluctuations in DA levels, as compared to CTL. Thus the diabetic state may be related to altered DA release pattern and/or altered post synaptic responses to DA.


In two studies, freely moving male rats were observed after they had varying amounts of copulation followed by injection apomorphine (AP); 60 mg/kg; a dopamine agonist), or vehicle. As expected, APO increased erections and yawning in males that had no antecedent sexual contact. Surprisingly, three iterations of one ejaculation produced erections even in vehicle-treated males, which did not differ from APO-injected copulating males. Sexually exhausted rats had no APO-induced changes, a significant depression relative to the other groups. Copulation did not affect yawning in either study. Stretching was rarely seen and may not be a reliable component of the syndrome. We infer that ejaculation has a dopaminergic effect on the erectile component of the PE/SYS, mediated in part by changes in neurochemical activity that are specific to brain systems involved in erection, or at least do not include systems that regulate yawning. [Supported by HD-08933.]

447.10 RESERPINE-INDUCED ORAL DYSKINESIA IN RATS: EFFECT OF DOSE AND COMPARISON TO TETRAZEPINE. J. L. Neuwanger*, E. Canabalos, and D. A. Davis. Department of Psychology, Arizona State University, Tempe, AZ 85287-1100.

Repeated administration of reserpine (1 mg/kg) in rats produces a severe depletion of monoamines, and induces the development of oral dyskinesia that reaches a maximal level within 3 days. To examine whether lower doses of reserpine would result in a slower development of oral dyskinesia, rats were injected SC with 0, 0.01, 0.05, 0.1, and 1 mg/kg reserpine every other day for 60 days. Oral dyskinesia was measured 24 hr after injections by recording the incidence of tongue protrusions during 30 min. Rats treated with 0.01 mg/kg developed TPs within 3 days, whereas rats treated with the 0.05 mg/kg developed TPs after 10-20 days, and rats treated with 0.1 mg/kg had not developed TPs by 60 days of treatment. These findings suggest that a large dose of reserpine may result in a slower development of TPs, similar to the prolonged development of tardive dyskinesia in humans. Furthermore, we suggest that a severe depletion of monoamines induce neurochemical changes similar to those that underly tardy dyskinesia, but at an accelerated rate. We also examined whether tetrazepam, another monoamine depleting agent, would induce oral dyskinesia. Rats were injected with either vehicle, reserpine (1 mg/kg, SC, every other day), or tetrazepine (3 mg/kg, SC, either once or twice daily for 21 days). TPs, grooming, and locomotor activity were measured on days 30-32 after the last injection. Both reserpine and tetrazepine treatment produced an increase in TPs. Tetrazepine, however, also produced an increase in grooming and locomotor activity relative to the other groups, suggesting the increase in TPs produced by tetrazepine may be due to an increase in overall activity rather than a dyskinesia. Reserpine produced a more severe depletion of monoamines (86-94%) relative to tetrazepine (28-58%). Reserpine also produced a 450-900% increase in dopamine and serotonin turnover as measured by neurochemical metabolite ratios, whereas tetrazepine did not alter turnover. Relationships between changes in brain monoamine levels and behavior will be discussed.


We have previously shown that the activity of schedule-induced polydipsia (SIP) was persistently depressed in a bilateral symmetrical locus coeruleus lesions and ventral tegmental area lesions in succeesion. The hypothesis that central catecholaminergic neurons modulate SIP behavior is supported by findings that the changes in the overall dopaminergic system observed in SIP may be due to the changes in the dopaminergic system of the nigrostriatal, mesocorticolimbic, and mesolimbic systems. The current study assessed the regional turnover of monoamines in SIP and control rats. It was found in the SIP that NA and DA levels and DA synthesis and utilization in the limbic area were increased and that NE synthesis in several pontine cerebrospinal-NF projections, e.g., subicampus, cortex and pons, were also increased. Conversely, the NE-metabolism in the hippocampus was decreased in SIP rats. In the same areas were decreased in the SIP rats. We therefore concluded that enhanced actions of both DA-limbic system and NE-cerebrospinal system are important in the process of SIP establishment.

447.12 DOPAMINERGIC CONTROL OF MALE CUMULATORY BEHAVIOR IN QUAIL. RECEPTOR AUTORADIOGRAPHY AND PHARMACOLOGICAL STUDIES P. A. L. W. D. E. Ball, I. M. G. and I. A. D. Laboratory of General and Comparative Biochemistry, University of Liège, Belgium, Dept. of Psychology, Johns Hopkins Univ., Baltimore, MD 21218, USA.

In quail, dopamine (DA) turnover in the medial preoptic nucleus (POM) is higher in males than in females. In the POM, is a key site of testosteron (T) on sexual behavior, this suggests that DA may be involved in the control of this behavior. Also, tyrosine hydroxylase-immunoreactive fibers innervate the preoptic area and hypothalamus. The behavioral effects of dopamine were analyzed by in vivo pharmacological manipulations. In a first experiment, the D1 agonist acid, apomorphine (AP), 10 mg/kg, did not change the behavior induced by T in castrated (CX) males. Inhibition was statistically significant for doses higher than 10 mg/kg and complete at 1 mg/kg. In a second experiment, repeated injections of AP (0.1, 0.01, 0.001 mg/kg) had no effect on the behavior, except for the dose of 1 mg inhibited the behavior in less performed 10 min after the injection. Several reports have indicated that AP can modulate sexual behavior in male rats. The inhibitory effects observed here may reflect a higher density or higher sensitivity of the D2 dopamine receptors in male rats compared to female rats. The effect of AP on the sexual behavior was measured using behavioral effects of AP in quail pretreated with apomorphine, a specific D2 antagonist. Apomorphine (0.03 mg/kg) should enhance sexual behavior and completely blocked the inhibitory effects of AP. These data suggest that sexual behavior in male quail is mediated through D2 receptors.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
448.1 HIGH-PEAK BLOOD EThANOL CONCENTRATIONS RESULTING FROM POSTNATAL EXPOSURE TO ETHANOL AFFECT MEMORY-BASED BUT NOT CUE-BASED DISCRIMINATIONS IN THE INFANT RAT. J. L. Diaz-Granados*, P. L. Greene, G. Y. Espinosa, & A. Ameral. Department of Psychology & Institute for Neuroscience, University of Texas, Austin, TX, 78712.

We have previously shown that postnatal exposure to ethanol that produces high-peak blood ethanol concentrations in infant rats, results in significant deficits in hippocampal neuroanatomy and discrimination learning based on the memory of singly alternated rewarded (R) and nonrewarded (N) trials (Greene, et al., Behav. Neurosci. 106:51, 1992). In the present experiment, we tested infant rats, artificially reared and exposed to ethanol from postnatal day 4 to 9, in a straight alley in a discrimination using rough and smooth floor textures as cues. On day 4 postpartum, pups were fed with gastric cannulas and a nutritionally adequate diet was infused intragastrically for 15 minutes every hour. In 4 consecutive morning feedings one group of pups received an ethanol-adulterated diet while postnatal controls received an isocaloric diet. (A third group was raised normally in a litter.) The remaining 20 feedings for all pups were with undiluted diet. On days 17-18, animals were tested on one of three discrimination schedules: single alternating S and N, random R and N, or random R and N followed by a discrimination reversal. The ethanol-exposed animals were not different from the normals or the artificially-reared controls in either alternating, or the random or reversed discriminations. These results indicate that the deficit in discrimination reported earlier in ethanol-exposed, hippocampally damaged animals is a memory deficit and not a general deficit in discrimination, since pups discriminate normally when external cues are made available. Supported by NIAAA grant AA07052.


Fetal Alcohol Syndrome is associated with altered sleep and feeding patterns in newborns. This experiment investigated persistent effects of in utero alcohol exposure on circadian rhythms in adult offspring. Male and female rats born to either control, pair-fed or alcohol-exposed dams were monitored for body temperature and activity using a telemetry system under various LD schedule conditions. Baseline (12:12 LD) or 14:10 LD measurements were affected by prenatal treatment, but females were more active, had higher mean body temperature, and had higher maximal temperatures that peaked earlier than males. Under a free-running schedule (LL), alcohol-exposed males were less active than controls; females did not differ by treatment. Mean and maximum temperatures were higher in alcohol-exposed males and lower in alcohol-exposed females than same sex controls, thus no longer displayed the typical sex-dependent pattern. Although all subjects demonstrated phase advancement, alcohol-exposed subjects lagged behind controls. Chronometric analysis of the volume of the SCN was inconclusive. These results suggest that the stress of a free-running schedule revealed underlying thermoregulatory dysfunction caused by prenatal alcohol exposure. (Supported by NSDA AA088050)

448.5 ETHANOL CONSUMPTION SUPPRESSES IMMUNE RESPONSE IN FETAL ETHANOL-EXPOSED RATS. J. Weiberg* and T.B. Jerpells, Dept of Anatomy, Univ of British Columbia, Vancouver, BC V6T 1Z3, Dept of Cellular Biology & Anatomy, Louisiana State Univ, Shreveport, LA 71130.

Ethanol consumption during pregnancy may alter immune function of offspring. Our previous work found that fetal ethanol-exposed (E) animals had decreased thymus number, decreased splenic lymphocyte response to concanavalin A (Con A), and a functional defect in T-cell response to a secondary stimulation by IL-2, as compared with pair-fed (PF) and control (C) animals. Importantly, deficits occurred in E males but not females. In the present study we examined the vulnerability of E offspring to the immunosuppressive effects of ethanol exposure in adulthood. Adult Sprague-Dawley males and females from prenatal E, PF and C conditions were assigned to E or PF adult treatment groups. Six groups were thus formed, with Prenatal–Adult treatments as follows: E–E, E–PF, PF–E, PF–PF, C–E, C–PF. Adult E or PF treatment was continued for 4 wk.

Sex differences in treatment effects were found. For females, C–PF animals had higher thymus and spleen weights than other groups. There were no differences among females from the 3 prenatal groups in splenic lymphocyte response to Con A, although adult ethanol consumption had some suppressive effects on responses in E and C animals. For males, thymus weights were lower in E–E than in E–PF animals; no other treatment groups showed this differential response. In addition, both E–E and E–PF males exhibited a suppressed lymphocyte response to Con A; males from prenatal PF and C conditions showed no suppression unless exposed to ethanol in adulthood. Supported by AA07789 (JW) and AA07731 (TR).


Learning and memory deficits after prenatal alcohol exposure occur both during development and in adulthood. Sleep deficits are also prominent in neonates and rats. Data from our previous findings demonstrate that paradoxical sleep and memory are correlated in several mammalian populations, suggesting that the possibility that deficits in paradoxical sleep will accompany deficits in memory in adult rats prenatally exposed to alcohol. We address this issue, pregnant Sprague-Dawley rats were fed a liquid diet including 35% ethanol-dereived calories, or were pair-fed an isocaloric diet from gestation days 8-19. Sleep (0h) records via continuous EEG and EOG, revealed a decrease in paradoxical sleep and an increase in stage 2 sleep. To address this issue, pregnant Sprague-Dawley rats were fed a liquid diet including 35% ethanol-dereived calories, or were pair-fed an isocaloric diet from gestation days 8-19. Sleep (0h) records via continuous EEG and EOG, revealed a decrease in paradoxical sleep and an increase in stage 2 sleep.

448.6 ACUTE ETHANOL EXPOSURE DEPRESSES STIMULATED GLUTAMATE RELEASE IN THE HIPPOCAMPUS OF THE DEVELOPING GUINEA PIG. J. P. Reynolds, J. V. Milligan, and W. B. Yager, Dept. of Pharmacology and Toxicology, and Dept. of Physiology, Queen's University, Kingston, Canada, K7L 3N6.

It has been proposed that glutamate (Glu) mediates ethanol (E) teratogenesis in the hippocampus. Our objective was to elucidate the effect of acute E exposure on spontaneous and potassium ion (K+)-stimulated release of Glu in the hippocampus of the near-term fetal and adult guinea pig. GLU release was measured as efflux in 300-μm thick, transverse, hippocampal slices. Acute in vivo E exposure involved oral intubation of 4 g E/kg body weight in near-term pregnant and adult male and female guinea pigs, which were sacrificed 2 hr after dosing. Acute in vitro E exposure involved incubation of tissue slices with 48 mM E, the apparent maximal blood [E] at 1 hr after 4 g/kg body weight. E did not affect spontaneous Glu efflux. In vitro E decreased K+-stimulated Glu efflux in the fetus and adult. In vivo E and in vitro E decreased K+-stimulated Glu efflux in the fetus, but not in the adult. These data indicate that (a) in the near-term fetal guinea pig, acute E exposure decreases stimulated Glu release in the hippocampus, and (b) in the adult guinea pig, tolerance develops to the depressant effect of E on stimulated hippocampal Glu release after a single E dose. (Supported by the MRC of Canada)
448.7 THIAMINE-DEPENDENT ENZYME CHANGES IN THE BRAINS OF ALCOHOLICS: RELATIONSHIP TO THE WERNICKE-KORSAKOFF SYNDROME. R.F. Butterworth, 1, 2 Kipf and C. Harper, 3 Neurosciences Research Unit, A-V CRC Hospital, St. Luc, University of Montreal, Montreal, Quebec, Canada H3X 3J7 and Dept of Psychiatry, University of Sydney, Sydney, Australia.

Previous studies in experimental animal models suggest that the cerebral dysfunction and cell death in the Wernicke-Korsakoff Syndrome (WKS) results from reductions of thiamine-dependent enzyme activities in brain (Butterworth, Alcohol & Alcoholism, 23, 271-279, 1989). As part of a series of studies of the role of thiamine in WKS in humans, activities of the thiamine-dependent enzymes pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (αKGDH) and transketolase (TK) were measured by standard spectrophotometric procedures in samples of frontal cortex and cerebellum of 10 alcoholic patients who died in hepatic coma with no clinical or pathological evidence of WKS, 2 alcoholic patients with neuropathologically confirmed WKS and 10 control subjects free from neurological disease.

Reductions of TK (by 75%), PDHC (by 82%) and αKGDH (by 90%) were observed in the brains of WKS patients. Activities of the non-thiamine-dependent enzyme glutamate dehydrogenase were within normal limits in brain tissue in all alcoholic patients vs. WKS. Activities of all three thiamine dependent enzymes were unchanged in the brains of alcoholic patients dying in hepatic coma. These findings demonstrate, for the first time, a direct link between reductions in activities of thiamine-dependent enzymes and WKS in humans.

(Funded by The Medical Research Council of Canada).

448.8 NEUROAL AND ASTROCYTIC RECEPTOR CHANGES IN AUTOPSIED BRAIN TISSUE FROM ALCOHOLIC PATIENTS WITH HEPATIC ENCEPHALOPATHY. J. Lavoie*, G. Girard, D.K. Leong and R.F. Butterworth, Neurosciences Research Unit, Andre-Vallée Clin. Res. Centre, Hospital St-Luc (University of Montreal), Montreal, Quebec, Canada H3X 3J4.

As part of a study of neurochemical mechanisms in alcohol-related brain damage, neuronal and astrocytic binding sites for specific radioligands were measured in autopsied brain tissue from 9 cirrhotic alcoholic patients and an equal number of control subjects free from neurologic disease. Neuropathological examination excluded the presence of WKS in humans. Densities of GABA-Benzodiazepine receptors were observed using [3H]-muscimol, [3H]-flunitrazepam or [3H]-Ro 15-1788. Binding parameters for the gluta matic receptor ligands [3H]-kainate or [3H]-MK801 were likewise unaltered in the brains of alcoholics. Densities of muscarinic cholinergic receptors, evaluated using [3H]-quinuclidinyl benzilate ([3H]-QNB) were increased by 48% in frontal cortex of alcoholic patients. Similar changes in [3H]-QNB binding were observed in rat brain following portacaval anastomosis (Giguere et al., Brain Res. 562, 1992) suggesting that these changes result from chronic liver disease rather than alcohol per se. These findings suggest that (i) neuronal loss in alcoholic brains in hepatic coma is restricted to a modest loss of cortical cholinergic neurons and (ii) chronic liver disease results in increased binding of [3H]-quinuclidinyl benzilate, benzodiazepine and muscarinic cholinergic receptors.

(Funded by The Medical Research Council of Canada)

448.9 CHRONIC INTRAGASTRIC INFUSION OF ETHANOL RESULTS IN THE PRODUCTION OF TOLERANCE TO ETHANOL IN MICE. W. Cao, T.K. Landa, A.C. Collins, Institute for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80303.

The present study characterized chronic intragastric infusion as a method for developing tolerance to ethanol in mice. The selectivity bred long sleep (LS) and short sleep (SS) mice were used. Male mice were anesthetized and were surgically prepared with a catheter in the stomach. The mice were infuse d every 6 hours with physiological saline or ethanol (3.0 or 4.0 g/kg). Potential tolerance to ethanol was determined by measuring the effects of challenge doses of ethanol on body temperature, open field and Y-maze activities. In addition, the mice were given an anesthetic dose of ethanol (6.0 g/kg for SS, 3.5 g/kg for LS) and the duration of sleep times was measured 6 hours after termination of infusion. Chronic infusion resulted in tolerance to virtually all the effects of ethanol in both mouse lines. However, the LS developed tolerance more readily (after shorter periods of infusion) and to a greater degree. Chronic infusion did not result in changes in the rate of ethanol metabolism in either mouse line which suggests that this procedure results in pharmacodynamic tolerance to ethanol's effects. Thus, the intragastric infusion method, which allows for very precise regulation of dose and dose interval, is a useful technique for producing tolerance to ethanol in mice. Supported by AA-06391 and DA-00116.

448.10 FURTHER CHARACTERIZATION OF AN ANIMAL MODEL OF ETHANOL WITHDRAWAL "KINDLING". H.C. Becker and R.L. Hale, VA Medical Center and Medical University of South Carolina, Charleston, SC 29401.

We have previously demonstrated that animals exposed to ethanol (EtOH) for a total of 48 hrs exhibit more severe withdrawal seizures if exposure is divided into three 18 hr intoxication/6 hr abstinence cycles than when the 48 hrs of exposure occurs in a single bout. This pattern of results supports the "kindling" hypothesis of EtOH withdrawal in that repeated episodes of EtOH withdrawal result in a progressive intensification of withdrawal seizures. The present study was designed to further characterize this animal model of EtOH withdrawal "kindling" and examine whether such a "kindled" response is still evident when withdrawal testing is conducted following an additional intoxication bout. Adult male mice were chronically exposed to EtOH via intragastric infusion in vapor chambers for 40 hrs prior to withdrawal testing. Prior to this 40 hr bout of intoxication, one group (Multiple Withdrawal; MW) received 3 cycles of 16 hrs EtOH exposure separated by 8 hrs periods of abstinence; a second group (Continuous Exposure; CE) received the same total EtOH exposure (48 hrs) without interruption; and a third group (Single Withdrawal, SW) did not receive any EtOH exposure prior to the 40 hr test cycle. A control (C) group was included that did not receive any EtOH exposure throughout the experiment. Blood EtOH concentrations (BEC) following the 40 hr period of EtOH exposure were 100-140 mg/dl for all EtOH-exposed groups. Mean areas under the 24 hr curve for handling-induced convulsions after the 40 hr EtOH exposure period for MW, CE, SW, and C groups were 33±5, 18±4, 3±1, and 0±0, respectively. These results indicate that differences in the severity of EtOH withdrawal seizures due to differences in prior withdrawal experience can be demonstrated even when later EtOH exposure patterns are equated. Supported by the VA Medical Research Service and NIAAA.

448.11 EFFECT OF ETHANOL ON ADENOSINE RECEPTORS CONTRIBUTES TO THE DEVELOPMENT OF THE WITHDRAWAL SYNDROME. S.M. Reszka, C.J. Walters, and J.L. Lal, Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX, 76107.

It has been hypothesized that stimulation of adenosine receptors is involved in ethanol (ETH) intoxication and the development of tolerance. We investigated an ethanol withdrawal symptom ("asthenia-like" behavior) using the elevated plus maze (EMP) paradigm (Laï et al., Alcohol 8, 687, 1991). Long-Evans hooded rats were given a balanced liquid diet containing 12% ETH for 40 hrs. After this period, animals were allowed to show a base line EMP response (CPT, 0.05-0.16 mg/kg, ip) during the last 6 days of ETH diet administration. Twelve hours after a final ETH dose (3g/kg, po), rats were tested in the EMP. We observed a significant reduction in the open-arm activity and an increase in the ataxic responses indicative of ETH withdrawal. Pair-fed treatment with an A1 adenosine agonist, R-(+)-N6-(2-phenylisopropyl)-adenosine (PA, 0.08-0.32 mg/kg, ip, 15 min), had little effect on performance in the EMP during ethanol withdrawal. Acute treatment with an A1 adenosine antagonist, 8-cyclopentyl-1,3-dimethylxanthine (CPT, 0.02-0.16 mg/kg, ip, 60 min), exacerbated ETH withdrawal with a further reduction in open-arm activity. Chronic treatment with CPT (0.04-0.16 mg/kg, ip) during the last 6 days of ETH diet administration resulted in a dose related increase in the amount of time spent in the open-arms of the EMP, but had little effect on the number of total arm entries. These data support the hypothesis that an ethanol stimulated increase in adenosine receptor activity may be associated with the development of dependence and that blockade of adenosine receptors during ethanol withdrawal will attenuate the display of "asthenia-like" behaviors during ethanol withdrawal. Supported by NIAAA Grant AA06980.


Studies examining post-training ethanol's effects on memory have found conflicting task-dependent results. Recent work suggests that this apparent contradiction may be due to an inherent aversive component of ethanol. According to this hypothesis, treatment with ethanol results in the loss of aversive information to the pretreatment stimulus complex, altering retentive performance. Low levels of chronic ethanol exposure using a helium-oxygen (heliox) gas mixture has been shown to antagonize a number of ethanol's acute and chronic behavioral effects. The present study investigated whether hyperbaric exposure antagonized ethanol's aversive effects on appetitive task memory. Male C57BL/6J mice were individually trained to find a cheese pellet. Mice were immediately injected i.p. with saline or 0.5, 1.0, or 2.0 g/kg ethanol (20% v/v). They were then returned to their home cage and exposed to 1 ATA air, 1 ATA heliox, or 12 ATA helium for 2 hours. Retention performance was measured 24 hrs after training in the non-drug state. Helium-air control mice ate the pellet significantly quicker after training, while all other mice exhibited a non-significant increase in latency, suggesting amnesia. However, habituation of locomotor activity and pellet approaches in all groups indicates that memory of the environment remained intact, while grooming data suggest that the amnesic response in the heliox and ethanol groups reflects a subtle aversive effect of these treatments. Collectively, it appears that post-training exposure to heliox, like ethanol, is aversive and does not impair memory storage processes. Further studies are necessary to determine the mechanism of heliox's aversive effect which may be related to hypobaric pressure that can antagonize the aversive effect of ethanol in this task. (Supported by NIAAA grants AA03972 & AA05234).
448.13
ANTAGONISM OF ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE BY THEOPHYLLINE. C.L. Cunningham*, D.H. Malott, P.O. Risberg. Oregon Health Sciences University, Portland, OR 97201-3098
Acute exposure to ethanol (EIOH) elevates adenosine levels in brain, and it appears that adenosine may be involved in the central depressant actions of EIOH. The present study examined the role of adenosine in mediating EIOH's rewarding effects by assessing the impact of a nonselective adenosine antagonist, theophylline, on ethanol-induced conditioned place preference. Intoxicated (BAC 2%) were exposed to a Pavlovian discriminative conditioning procedure in which a distinctive floor (tactile) stimulus (CS+) was paired four times with either EIOH (2 g/kg, IP), theophylline (30 mg/kg), or the combination of theophylline and EIOH. A different floor stimulus (CS-) was paired only with saline. Conditioning trial duration was 30 min and the theophylline pre-treatment interval was 30 min. Preference testing was conducted in the absence of either drug. Both EIOH and theophylline increased general activity on conditioning trials and their combination produced greater activity than either drug alone. As expected, EIOH alone resulted in conditioned place preference.
However, theophylline alone produced conditioned place aversion. The group receiving the combination of theophylline and EIOH showed no evidence of place conditioning, suggesting these drugs cancelled each other's hedonic effects. This outcome may not appear to represent a pharmacological antagonism, but seems more readily interpreted as an instance of behavioral or "functional antagonism."
[Supported by AA08621, AA07702, AA07468]

DRUGS OF ABUSE: STIMULANTS I

449.2
EFFECT OF ETHANOL AND GLUTATHIONE ON REACTION TIME IN RATS. Z.M. Post*, P.K. Randall, S.W. Leslie and C.K. Erickson. Dv. of Pharmacol., Coll. of Pharm., Univ. of TX, Austin, TX 78712
Ethanol (EIOH) and NMDA similarly stimulate calcium uptake into dissociated brain cells. However, ethanol (EIOH) inhibits only GSH-stimulated uptake, perhaps because GSH blocks its purported site of action, the glycine site on the NMDA receptor complex. The interaction between GSH and EIOH has proven to be behaviorally significant in the rat given a hypothetical dose of EIOH regained the righting reflex sooner when pretreated with intracerebroventricular (ICV) GSH. The purpose of this study was to determine whether GSH pretreatment would also lessen EIOH-induced impairment of a reaction time (RT) task in rats. On week 1 of a simple crossover design, behaviorally tolerant rats (n=12) were administered EIOH (i.g., 2.8 g/kg, 20% w/v in water) 15 min after ICV GSH (10 µl, 20 mM) or vehicle (10 µl artificial cerebrospinal fluid); on week 2, EIOH followed the alternate ICV agent. Although EIOH by itself impaired normal speed and speed of response (85 v. 96% and 312 v. 240 ± 11 ms at 30 min, respectively), performance after GSH was no different from vehicle. In a second experiment, EIOH-naive rats (n=10) were given 2.4 g/kg EIOH after ICV GSH or vehicle. Again, EIOH alone impaired response (63% success and 453 ± 29 ms) but GSH-treated rats were not protected. This lack of effect is probably due, at least in part, to the fact that the animals are highly practiced by the time drugs are administered. Also, there is great incentive to perform successfully, and thus, compensatory behavior is developed rapidly. It would be appropriate to test a behavior of intermediate demand which measures motor control, but to which less behavioral tolerance to EIOH develops. (Supported by funds from the Univ. of TX.)

449.3
Effects of nicotine (1.5 mg, i.v.) on cerebral metabolic rates for glucose (CMRglc) are being studied in human volunteers, including cigarette smokers and nonsmokers. CMRglc is measured by positron emission tomography using the [15O] water methodology. Subjective and cardiovascular responses are also recorded.
Preliminary results indicated that smokers showed globally higher CMRglc than nonsmokers in the placebo condition, and that both groups showed a widespread decrease of about 10% in response to nicotine. Smokers gave higher subjective responses than nonsmokers to questions about drug liking (e.g., "Did the drug have good effects?") or "How much did you like the drug?"). There were no differences between smokers and nonsmokers on ratings of drug strength (e.g., "How strong was the drug effect?" or "How much did you feel the drug?"). Smokers showed more tachycardia than nonsmokers, presumably due to tolerance. The findings suggest that nicotine resembles other drugs of abuse in that it reduces CMRglc. Relationships between CMRglc and effects on mood are under investigation.

449.4
BEHAVIORAL EFFECTS OF NICOTINE CONSUMED BY RATS. C. Kair* and G. Mellor, Department of Psychology, University of Wyoming, Laramie, WY 82071
Rats that had not consumed water for 24 hr were placed into 41 x 41 x 30 cm clear acrylic chambers with a 10-ml drinking tube in one corner. Each rat remained in the chamber for one hour, and activity was recorded using an Omnitech monitor. Water was given in the home cages for 2 hr after each session. By the 7th day all rats drank all 10 ml of water in the test chamber within 5 min. On the 8th-14th days the drinking tube contained 10% sucrose with 10 µg/ml nicotine. At first there was no apparent effect on locomotor activity, but over the 7 days of nicotine testing the total distance moved during the hour increased more than threefold. Subsequent test days alternated between presentation of 10% sucrose alone or sucrose plus nicotine. A significant dose-effect relationship was found with locomotor activity increasing in response to 5, 10 and 20 µg/ml nicotine solutions. Pretreatment with 1.0 mg/kg mecamylamine 20 min prior to tests with 10 µg/ml nicotine had no effect on volume consumed or time to drink, but did reduce the locomotor response.
Rats will drink 75 ml/day of 10 µg/ml nicotine in 10% sucrose in 24 hr, 2-bottle drinking tests with plain water in the 2nd bottle. Those rats quickly stop drinking significant amounts of nicotine either when the sucrose is removed from the mixture or when a third bottle is presented containing 10% sucrose without nicotine. Thus, we have been unable to demonstrate that rats develop a dependence on nicotine after consuming this sucrose-nicotine mixture. These findings call into question theories of drug dependence that tie reinforcing properties of drugs to their locomotor stimulant effects.
449.5

There is evidence to suggest that increases in dopamine in the nucleus accumbens mediates the rewarding effects of psychomotor stimulants and that D2/D3 dopamine antagonists can reverse stimulant-induced changes in mesolimbic dopamine activity. Previous work from our laboratory has shown that MDL 28,133 (MDL) can attenuate the reinforcing effect of d-amphetamine and cocaine. The present study assessed the ability of MDL 28,133 to attenuate or block nicotine-induced facilitation of brain stimulation reward. Rats implanted with bipolar ventral tegmental area electrodes were administered 1.0, 3.0, or 6.0 mg/kg MDL (IP) 1 hr prior to testing. MDL alone did not produce any change in responding. However, when co-administered with nicotine (0.4 & 0.8 mg/kg IP, 45 min post-MDL), MDL produced a dose-dependent reversal of the threshold lowering effect of nicotine. Analysis of response rates indicated that MDL did not interfere with the animal's performance, as do other compounds with dopamine antagonist properties (i.e., haloperidol). This suggests that compounds that couple with a serotonergic component may be effective pharmacotherapies in the treatment of psychomotor stimulant abuse. This research was supported by NIDA NRS grant DA04849 to P.Z. Manderscheidt and NIDA grant DA04483 to R.A. Frank.

449.7
CAFFEINE AND NICOTINE DO NOT MAINTAIN SELF-ADMINISTRATION IN RATS. J. Robinson*, S.L. Varga, J. Broadbent, and S.I. Dworkin. Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157.

Although caffeine (CAF) and nicotine (NIC) are widely used, their reinforcing characteristics remain obscure. Attempts were made to assess the reinforcing function of these compounds. Six male Fischer 344 rats were implanted with indwelling jugular catheters and then trained on a fixed ratio (FR) schedule of food presentation. Animals were then allowed access to CAF (0.25 or 0.5 mg/ml) or NIC (10 µg/ml) on a FR 1 schedule. In a different paradigm, one rat was given access to food, water, and CAF (0.25 mg/ml) 24 hr/day on a concurrent FR 1, FR 3 schedule, maintained with CAF at the single-lever chamber. Results indicated a relatively high rate of responding following food-training, which substantially declined with time. CAF (0.25 mg/ml) infusions declined by approx. 75% in the second month as compared with the first. Increasing the dose (0.5 mg/ml) did not change response rates. Under the concurrent schedule only food and water maintained responding. These data indicate that CAF does not maintain reliable self-administration (SA) in rats, even when the animals were trained with food. It was found previously (Dworkin et al., Soc. Neurosci. Abs. 17, 425, 1991) that NIC did not engender and maintain SA which was delivered over a 5.6 sec time period. We examined if infusion duration could affect NIC SA. Rats allowed access to NIC did not attain reliable SA when infusions were delivered over 0.9 sec. These data confirm that NIC and CAF are not potent reinforcers in Fischer rats. (Supported by a contract from the R.J. Reynolds Tobacco Co.)

449.9
THE EFFECTS OF CAFFEINE ON CORTICAL AROUSAL. D.Velkonja, S.J. Segalowitz and S. Reins*, Dept. of Psychology, University of Waterloo, Waterloo, ONT, N2L 3G1 and Brock University, St. Catharines, ONT, L2S 3A1.

Individual differences in reactivity and tolerance to caffeine are predicted to play a critical role in cortical arousal, as measured by electrophysiological variables. Furthermore, it is believed that caffeine will have time-based effects on cortical arousal varying with daily patterns of activity and caffeine consumption. This hypothesis was tested in 20 first year university undergraduate students using a within-subjects design. Each subject participated in two test sessions, in which they consumed one half cup of naturally decaffeinated coffee or one containing powdered caffeine in doses based on the subjects body weight (3mg/kg). Subjects were blind to experimental conditions. They proceeded to complete questionnaires related to their reactivity and tolerance to caffeine, and whether they were morning or evening types. One half hour elapsed between their consumption of the beverage and EEG testing. Subject's reaction time performance, auditory P300s and visual CNVs were compared on the two occasions. Prior to their date of testing subjects were required to monitor their caffeine consumption over a two-week period in order to determine their habituation to caffeine. A general increase in the amplitude of both P300, and CNV was observed, as well as a decrease in reaction time variability, which we expect will be related to individual differences in the CNV. We also predict that an increase in the CNV will also be related to an increase in caffeine consumption. It is expected that caffeine consumers who are expected to have a higher tolerance and lower reactivity to the caffeine, and an increase in a P3 amplitude jitter in non-habitual caffeine consumers who are expected to have higher tolerance and higher reactivity to the caffeine.

449.10

Phencyclidine (PCP) has been considered to induce a schizophrenia-like psychosis in human and behavioral disturbances in experimental animals by blocking the N-methyl-D-aspartate (NMDA) receptor. This study was performed to elucidate the effect of intercerebrovascular (i.c.v.) infusion of different stereoisomers of PCP on NMDA receptor on PCP-induced hyperactivity in the rat. Drugs were infused 10min before PCP administration (10mg/Kg, intraperitoneally). The locomotor activity was evaluated based on the method of Schoen (1979) with minor modifications. In some experiments, the locomotor activity was also quantitated automatically with Animex-Auto (Muranoichi-kikai Co., Japan). D-Alanine and D-serine significantly reduced the PCP-induced hyperactivity in a dose dependent manner. In contrast, the L-isomers of these amino acids were much less effective in antagonizing the hyperactivity induced by PCP. The stereoisomer of the D-enantiomer, that the antagonism of behavioral effects of PCP may be due to activation of the striamo-thalamo-cortical (STC) circuit. These findings that the stimulation of the allatostatin regulation site of the STC system might play a therapeutic effect in schizophrenia attributed to PCP and schizophrenia.
549.1


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Phencyclidine (PCP;angel dust) is a drug of abuse known to produce a behavioral state in humans resembling schizophrenia/psychosis. PCP is a noncompetitive NMDA receptor antagonist, and produces a variety of behavior in rats including circling, however, the behavioral effects of other noncompetitive NMDA receptor antagonists such as (+) MK-801 or TCP are still being elucidated. Here, adult female rats were dosed with PCP (10 mg/kg,ip), (+) MK-801 (0.1 mg/kg,ip) or TCP (1 mg/kg,ip) on two different occasions, one day before sacrifice to determine monoamine levels by HFLC/EC. Animals injected with PCP, (+) MK-801 or TCP showed a preference to turn to the left (65%, 72%, 62% respectively). PCP and (+) MK-801 also produced a significant increase of DOPAC and HVA in whole caudate nucleus (CN), nucleus accumbens and olfactory tubercles in both sides of the brain. Further dissection of the CN into medioventral and dorsolateral revealed that HVA was increased bilaterally except in globus pallidus where we found significant increases in dopamine (DA), DOPAC and HVA only on the left side after PCP and (+) MK-801 administration. However, after TCP treatment the effects were similar but did not reach statistically significant levels. These data suggest that PCP, (+) MK-801 and TCP produce a greater preference to turn left than right, a finding similar to that found in human psychoses. Furthermore, it is possible that this preference is due to the high concentrations of DA, DOPAC and HVA found in the left side of the globus pallidus after drug administration.

549.13


Sensation-seeking is a human personality trait associated with drug use, compulsive sexual practices, and a history of the seeking situations commonly considered stressful. A common feature of such situations is activation of the hypothalamus-pituitary-adrenal axis leading to corticosterone secretion. Since, glucocorticoids have euphoric effects in certain individuals and increase the reinforcing properties of drugs in animals, a higher sensitivity to the reinforcing effects of glucocorticoids might be a biological basis for sensation-seeking. In order to answer this question, we have studied the reinforcing properties of corticosterone, by means of intravenous self-administration, and the effects of this hormone on the mesolimbic dopaminergic (DA) system, by means of in vivo microdialysis. The DA system has already been studied because it is considered the main substrate of drug reinforcement. The experiments show that: i) corticosterone, in the range of stress levels, induces intravenous self-administration and increases DA release in the nucleus accumbens; ii) animals that, similar to high sensation-seekers, are more responsive to novelty and more prone to self-administer drugs, are also more sensitive to the reinforcing effect of corticosterone and have a higher corticosterone-induced dopamine release in the n. accumbens. These results suggest that different sensitivity to corticosterone's reinforcing effects may be a biological basis for the individual differences associated with sensation-seeking. Furthermore, individual differences in corticosterone-induced dopamine release may be the neural substrate of different sensitivity to corticosterone's reinforcing properties. These findings provide new insights on the physiology of glucocorticoids and, since these hormones influence drug self-administration as well as immune functions, these results may also be relevant to clinical practice.

549.12

SIGMA-INDUCED INHIBITION OF ADRENOMEDULLARY [3H]NA RECEPTER REUPTAKE IS NOT MEDIATED BY THE ANTIDEPRESSANT SITE BOUND BY [3H]DESIPRamine. C. Rogers and S. Lemaire, University of Ottawa, Ottawa, Ontario Canada K1H 8M5. The ability of several a ligands to inhibit uptake of noradrenaline has been examined previously (Massamiri, 1991; Rogers, 1991). At present, the mechanism(s) involved in this inhibition are unknown. Evidence has been presented to suggest that a ligands block noradrenaline reuptake by interaction with [3H]desipramine ([3H]DMI) receptors. The purpose of this investigation was to determine the degree of interaction of a ligands with [3H]DMI binding sites in bovine adren medulla. [3H]DMI binding was dependent upon Na+, protein concentration, time, temperature, and was saturable (Kd=2.87 nM Bmax=216 pmol/g protein). [3H]DMI binding was displaced by a ligands, although the rank order of potency of these ligands did not parallel that found in [3H]NA reuptake assays in this tissue. This data suggests that a ligands and desipramine inhibit [3H]NA reuptake through distinct receptors in bovine adren medulla. This work was supported by the HSFG, C.R. is a HSFG scholar.
450.3

BASAL DOPAMINE LEVELS IN THE NUCLEUS ACCUMBENS ARE DECREASED DURING COCAINE WITHDRAWAL AFTER UNLIMITED-ACCESS COCAINE SELF-ADMINISTRATION. E. Wills*, A. Markou, and G.P. Koob. Department of Neuropharmacology, Research Triangle Institute, La Jolla, CA 92037.

Clinical observations indicate that chronic cocaine abuse is followed by a withdrawal syndrome resembling an episode of major depression. As analogous to the withdrawal syndrome associated with "post-cocaine depression" characterized by significant elevations in brain reward thresholds, a decrease in dopamine levels has recently been reported in rats administered the NAC during periods of intravenous cocaine self-administration (Markou & Koob, Neuropharmacology, 1991). This withdrawal-associated reward deficit may involve a specific impairment in the ability to experience anhedonia in the region of the nucleus accumbens (NAC). To test this hypothesis we have monitored extracellular DA levels in the NAC by intracranial microdialysis in awake rats following dopamine withdrawal from cocaine after periods of unlimited-access, intravenous self-administration (9.5 to 21.75 hours). Cocaine withdrawal was associated with significant reductions in basal DA overflow that persisted up to 10 hours. Maximal inhibition of DA release (Mean ± S.E.M.: 66.15 ± 3.33 percent of basal levels) was observed at 4-6 hours after termination of cocaine self-administration and was positively correlated (r = 0.935) with the duration of the preceding self-administration episode. The results suggest that suppression of basal DA release in the NAC is a direct adaptive change in chronic cocaine exposure and may constitute an important neurochemical correlate of the cocaine withdrawal syndrome as measured by brain stimulation reward thresholds.

450.5

INCREASES IN EXTRACELLULAR BIgenic AMINE CONCENTRATIONS FOLLOWING COCAINE OR HEROIN SELF-ADMINISTRATION S.E. Hembry*, T.J. Martin, C.C., B. O'Dowd, & J.E. Smith. Center for Neurobiology of Drug Abuse, Dept. of Phys./Pharm., Bowman Gray School of Medicine, Wata Forest Univ., Winston-Salem, NC 27157.

Nuclear accumbens dopamine is believed to mediate the reinforcing effects of psychotropic compounds. Cocaine self-administration increases extracellular DA levels in the NAC in a dose-dependent manner (Petit & Justice, 1989, PIB, 34, 859-904). Response-independent administration of opioids enhances extracellular dopamine concentrations although this effect has not been demonstrated during response-dependent administration. The present study was initiated to determine whether extracellular dopamine concentrations in the NAC are elevated during heroin self-administration in a similar manner to that observed during cocaine self-administration. Responding was maintained by either intravenous infusion of heroin (60 or 1000 μg/kg/injection) or opioids (3 mg/kg/injection). Following a minimum of twenty days stable responding, microdialysis probes were inserted through previously implanted cannula. The following days, control samples were collected for at least five minutes throughout the entire session. HPLC coupled to electrochemical or ultraviolet detection was used to assay the dialysate samples for catecholamines and cocaine, respectively. Cocaine self-administration increased extracellular NACC dopamine and serotonin although metabolite concentrations did not significantly differ from baseline.

In addition, preliminary results suggest that heroin self-administration produced significant elevations in dopamine comparable to the relative increase observed with cocaine. (Supported in part by USPHS Research Grants DA-01819, DA-03628, and DA-06634)

450.7

EFFECTS OF DAILY MULTIPLE INJECTIONS OF COCAINE ON THE DOPAMINERGIC SYSTEMS IN RATS: AN IN VIVO MICRODIALYSIS STUDY. E. Gardner and M.J. Koob, The Rockefeller University, New York, NY 10021.

The aim of this study was to investigate the response of the dopaminergic system to a pattern of drug administration that mimics cocaine users' binges. Rats received three i.p. injections of cocaine (3 x 10 mg/kg) or saline (3 x 1 ml/kg) over two-hour periods. On day 14, cocaine was injected for the first time and the tissue continued to receive cocaine injections and the time course of dopamine (DA) and its metabolites were measured in the nucleus accumbens and in the striatum using in vivo microdialysis. In both regions extracellular DA basal levels were not significantly different in saline versus cocaine, 0.060 nM versus 2.56 ± 0.53 nM in the nucleus accumbens, p<0.06, 9.32 ± 2.4 nM versus 7.11 ± 1.42 nM in the striatum, p<0.02; n=5).

Acute cocaine administration led to an increase in extracellular DA levels in both regions that peaked after the second cocaine injection (3.5 times greater than basal levels in the nucleus accumbens, 3 times in the striatum). Chronic treatment reversed the pattern observed in the acute condition. The peak in DA levels was achieved only after the third injection, and in the striatum, in contrast to the nucleus accumbens, the extracellular DA concentrations were still elevated at the maximum acute challenge. The present results differ from the conclusions of studies involving a single daily cocaine injection where DA levels were reported by chronic exposure. Therefore the pattern of cocaine administration appears to be a major factor in the response of the dopaminergic systems. (Supported by the Aaron Diamond Foundation and DA-P50-06130)

450.4

REVERSE TOLERANCE OF SEROTONIN AND DOPAMINE IN THE NUCLEUS ACCUMBENS AND DORSAL RAPHE NUCLEUS INDUCED BY CHRONIC COCAINE TREATMENT. L.H. Parsons* and J.B. Justice, Jr., Dept. of Chemistry, Emory University, Atlanta, GA 30322.

The effect of chronic cocaine administration on the response to a cocaine challenge was examined for serotonin (5-HT) and dopamine (DA) in the nucleus accumbens (N ACC) and dorsal raphé nucleus (DRN) of the rat using a dual probe in vivo microdialysis procedure. Rats were treated for ten days with cocaine (20 mg/kg, ip; n=5) or saline (0.9% saline, 10 ml/kg, ip; n=4). and were euthanized 10 mg/kg ip. injection of cocaine on the test day (day 11). Based on maximum percent increases in cocaine challenge, DA doses were chosen to induce a similar acute response to cocaine in both the N ACC and DRN, though there was no significant interaction between region and degree of sensitization. 5-HT was also significantly sensitized in both regions of chronically cocaine treated rats. A significant interaction between region and degree of sensitization, indicating that the cell body region produced greater 5-HT sensitization than the N ACC terminal field. Both the N ACC and DRN were obtained from the same probe in a given region for each animal. DA-5HT area under the curve (AUC) ratios were used to compare the sensitization of DA relative to 5-HT. In both regions there were reduced DA-5HT AUC ratios in chronic as compared with acute animals, though this reduction reached significance only in the DRN. This suggests that the 5-HT system is relatively more sensitive to chronic cocaine administration than the DA system.

Additionally, the interaction between 5-HT and DA was assessed by adding various concentrations of 5-HT to the perfusate of a microdialysis probe in the N ACC and observing the concurrent change in dopamine. DA-5HT was found to concentration-dependently increase dopamine, with peroxide 5-HT concentration-borne (0-400 nM). These concentrations were found to be effective in decreasing the effectiveness by the relatively non-specific 5-HT antagonist pindolol, the specific 5-HT2 antagonist LY 53877, and the relatively specific 5-HT3 antagonist MDL 7222. These results indicate that 5-HT4 enhances extracellular DA levels in the N ACC.

450.6

60-DAY CHRONIC COCAINE TREATMENT DESENSITIZES EXTRA Cellular DOPAMINE OVERFLOW IN CAUDATE-PUTEAMEN BUT NOT IN NUCLEUS ACCUMBENS. J. Chen*, R. Marmor, E. Rossebuen, W. Parsons and E.L. Gardner, Departments of Pharmacology and Neuroscience, Albert Einstein College of Medicine, New York, NY 10461.

Rats with in vivo brain microdialysis probes in the nucleus accumbens (Acc) and caudate-putamen (Cpu) were treated with cocaine (10 mg/kg/day) for 5, 30 and 60 days. 24 hrs after the last cocaine injection, rats of each group were challenged with the same dose of cocaine and extracellular dopamine (DA) overflow in Acc and Cpu measured by in vivo microdialysis. In both Acc and Cpu, biochemical sensitization of extracellular DA overflow was not seen in rats given repeated cocaine for 5 days. In contrast, 30 days of chronic cocaine did robustly augment the enhanced extracellular DA overflow produced by the challenge dose of cocaine. In animals given 60 days of chronic cocaine, striking decreases in DA overflow in both Acc and Cpu manifested themselves. In Acc, the augmented extracellular DA overflow to cocaine challenge seen after 60 days of cocaine was identical to that seen after 30 days of cocaine. In Cpu, after 60 days of chronic cocaine, the enhanced extracellular DA overflow produced by cocaine challenge was identical to that seen with no prior cocaine treatment. Thus, in Acc neurochemical sensitization to repeated cocaine occurred, and the sensitization maintains itself, while in Cpu cocaine sensitization occurs, but then some compensatory mechanism comes into play to produce a neurochemical desensitization to cocaine. This suggests that cellular mechanisms underlying cocaine sensitization differ between the mesolimbic and mesostriatal DA systems. (Supported by a research grant from the Aaron Diamond Foundation.)

450.8


In vivo electrochemistry was used to investigate the mechanisms contributing to the clearance of locally-applied dopamine (DA) in dorsal striatum and nucleus accumbens of urethane-anesthetized rats. Chronic in vivo neurotransmitter and dopamine transporter (DAT) noramperometric recordings were continuously made at 5 Hz using Nafion-coated carbon fiber microdisc. Chronic administration of cocaine (12-100 nl, 200 μM barrel concentration) was pressure ejected at 5-s intervals from a microcapillary positioned 280 ± 30 μm from the electrode, transistors and reproduced increases in DA (0.5-4 μM) were detected. Substitution of α-methyl-DA, which is a substrate for the DA transporter but not for monoamine oxidase, for DA in the microcapillary did not substantially alter the time course of the resulting signals. These results indicate that metabolism of locally-applied DA to dihydroxyphenylacetic acid is not responsible for the decline in the DA signal. Similarly, changing the applied oxidation potential from 0.45V to 0.80V, which allows detection of 3-methoxytyramine formed from DA via catechol-O-methyltransferase, did not affect the signal amplitude and time course. In contrast, local application of the DA uptake inhibitor cocaine or nomifensine (800 μM barrel concentration, applied 20-60 sec before DA) significantly increased the amplitude and time course of the DA signals in both brain regions, a result similar to that observed with systemic administration of cocaine. These results indicate that uptake of DA by the neuronal DA transporter, rather than DA metabolism, is the major mechanism for clearing locally-applied DA from the extracellular space. (Supported by USPHS DA04216, DA0174 & NS08199 and NSF BNS-9110308)
450.9
We investigated whether differential changes in the dopamine (DA) transporters in the nucleus accumbens (NAc) of a parametrically trained and doral striatum could be involved with cocaine-induced behavioral sensitization by monitoring the clearance of locally applied DA in anesthetized rats using in vivo electronbeam autoradiography. We given daily injections of cocaine (10 mg/kg i.p.) or saline for seven days, withdrawn for seven days, and prepared for electron-beam recording. When a fixed amount of DA (2.5-10 fmol) was pressure-ejected at 5 min intervals from a micropipette positioned 270 ± 30 μm from the recording electrode, transient and reproducible decrease in extracellular DA was detected. In response to a challenge injection of cocaine (10 mg/kg i.p.), the signals in the NAc of the cocaine-treated rats became prolonged and the clearance rate of DA decreased, indicating significant inhibition of the DA transporter. In contrast, in the striatum there was a transient increase in the DA clearance rate. In saline-treated animals, the signals from both regions were similar to signals from untreated animals given an acute injection of cocaine (10 mg/kg i.p.) or saline. Quantitative autoradiography with 3H-mazindol revealed that the affinity of the DA transporter for cocaine and the density of binding sites were similar in cocaine- and saline-treated rats. Behaviorally, 50% of the cocaine-treated animals were sensitized; however, both sensitized and unsensitized animals displayed similar changes in DA clearance rate. Nonetheless, the observed decrease in DA clearance rate in the NAc of the cocaine-treated rats is consistent with increased DA transmission in response to cocaine challenge. Supported by USPHS DA04216, NS0199 & NSF BNS-910308.

450.11
EFFECTS OF INTRA-ACCUMBENS AND INTRA-AMYGDALOID SCH23390 ON INTRAVENOUS COCAINE SELF-ADMINISTRATION IN THE RAT. A. McGregor and D.C.S. Boulton*. Life Sciences Research Centre, Carleton University, Ottawa, K1S 5B6, Canada.
The nucleus accumbens (NAc) and its heavy dopaminergic [DA] innervation are generally considered to be a neural substrate pivotal in mediating the reinforcing effects of psychostimulants. Drugs, however, the amygdaloid complex is another neural site receiving a substantial DA innervation that has been relatively overlooked with respect to mechanisms of drug abuse. This work investigated the possible involvement of the amygdaloid complex (AMY) in cocaine self-administration (0.6mg/kg/Inj) under a fixed ratio schedule (FR1) of retrograde compared to that of the NAc. Bilateral injections of the D1 antagonist, SCH23390, were made directly into the NAc or AMY. The results of both the significant close dependent (1μg] - 2.0μg/side/0.5μl) Increase in the rate of self-administration was produced, demonstrating that both sites make a contribution to cocaine reinforcement mechanisms. In addition however, blockade of the D1 receptor within the AMY produced a two-fold increase in the rate of cocaine intake with respect to that produced within the NAc. These results suggest that in addition to the NAc, the AMY also has a contribution to make to cocaine reinforcement mechanisms. (Supported by the MRC of Canada).

450.13
Using quantitative autoradiography, the influence of unlimited cocaine self-administration (28 weeks on binding to the dopane (DA) uptake) [3H]DA (12,935 displaced with 1 μM mazindol) and cocaine acceptor (10 nM [3H]WIN 35,428 displaced with 30 μM cocaine) sites in rat brain was examined. In cocaine in t.e. 100% selective on cocaine acceptor binding. [3H]WIN 35,428 to the DA transporter in striatum, n. accumbens (nacal, substantia nigra and ventro-lateral tegmental area) was unaltered relative to controls (n=5). However, specific binding of [3H]WIN 35,428 to the cocaine acceptor sites was significantly elevated (p<0.05) in striatum (+63%) and n. accumbens (+31%). Following 3 weeks withdrawal from unlimited cocaine self-administration specific binding of [3H]DA took 12,935 was unaltered whereas binding of [3H]WIN 35,428 was significantly reduced (p<0.05, n= in striatum (-93%), n. accumbens (-51%) and VTA (-26%). A unique observation of unaltered binding of [3H]WIN 12,935 3 weeks after the last administration of cocaine, suggests that there is no permanent degeneration of dopamine neurons. The present study demonstrates that cocaine self-administration produced an increase in the density of the cocaine acceptor binding site which was retained following withdrawal and suggests that the cocaine acceptor binding site is not simply a functional expression of DA or to the presence and subsequent absence of cocaine, which acts like an antagonist at the DA transporter. Altered affinity/density of the cocaine acceptor could be important to establish whether the functional significance of such changes following chronic cocaine exposure and during the course of withdrawal. (Supported by NIDA grant DA07182.)

450.10
EFFECTS OF SPECIFIC BRAIN AMINE DEPLETING LESIONS ON COCAINE-INDUCED CONDITIONED INCREASES IN LOMOCOMUTER OUTPUT. A. Pett*, D.N. Thomas and R.M. Post*. Biological Psychiatry Branch, NIH, Bldg. 10, Room 31222, Bethesda, MD 20892.
Environmental stimuli paired with cocaine acquire the ability to elicit increases in locomotor behaviour and potentiate the actions of cocaine during subsequent presentations. We mixed a single and effective dose of the neuronal substrates which underlie the conditioned effects of cocaine. On DAY 1, rats were injected with 3.5mg/kg of cocaine (PAIRED) or saline (SINGLE) followed by injection of saline the next day. On DAY 2, all rats were injected with saline prior to cocaine injection. In the locomotor chamber for 30min. The following return to the home cage animals that were injected with 30mg/kg cocaine, while those that were not were injected with saline. On DAY 2, all rats were injected with 30mg/kg cocaine prior to placement in the locomotor chamber for 30min. The locomotor activity activity is revealed by significant increases in locomotor output of the PAIRED versus the UNPAIRED group on DAY 2. We have previously found that 5-OHDA lesions of the nucleus accumbens prevent the development of such conditioning with cocaine. The purpose of these studies was to evaluate the role of the n. accumbens and dorsal raphe with 5-OHDA or in the median and doral raphe with 5-HT. Controls groups were sham lesioned. One week following surgery, the animals were conditioned and tested as described above. Neither frontal cortex nor striatal lesions had an effect on the acquisition of conditioned locomotor activity, although the striatal lesion did seem to attenuate the conditioned stereotypy. Lesions of the locus coeruleus or raphe had no effect on cocaine-induced conditioning. These findings suggest that the critical dopaminergic neural substrates for cocaine-induced conditioned locomotor behaviour are the amygdala and the nucleus accumbens. Serotonergic and noradrenergic pathways do not appear to play a role in cocaine-induced conditioning in our paradigm.

450.12
One model used to examine individual differences in drug preference uses the inbred Fischer 344 and Lewis rat strains, which show differential oral self-administration of carbohydrates, fats, proteins, and alcohol. We have shown inherent biochemical differences in reward-implicated sites between these strains, with an upregulated dopamine (DA) system in the nucleus accumbens and the ventral tegmental area (VTA) of the drug-prefering Lewis rat (Beltner-Johnson et al., Brain Res. 681, 147-191; Gultart et al., proc. in press). We have also demonstrated strain differences in other drug-related behaviors: compared to Fischer rat, Lewis rat shows greater locomotor sensitization following amphetamine and cocaine, and more readily acquire intravenous cocaine self-administration.
We have also begun to examine similar individual differences in drug sensitivity. The role of drug self-administration in these results was reviewed. Rats were assessed for locomotor activity in a novel environment, and the highest (HR) and lowest (LR) responders were selected for biochemical analysis. Compared to HR rats, LR rats showed higher levels of DA-dependent protein kinase in the NAc, and of TH in the VTA. We are currently testing HR and LR rats for the acquisition of cocaine self-administration.
Such integrated biochemical and behavioral assessments should enable the identification of some of the biochemical factors that contribute to individual genetic vulnerability to drug addiction.

450.14
Withdrawal From Continuous or Intermittent Cocaine: Dopamine Autoreceptor Sensitivity. KUNG, G.R., KUHN, C. AND ELLINWOOD, E.H.* JR.
Previous research in this laboratory indicates that daily intermittent injections of cocaine result in an enhanced dopamine (DA) response in caudate-putamen brain slices, to different cocaine concentrations. In contrast, the continuous infusion of equivalent daily doses of cocaine result in an attenuated DA response in caudate-putamen brain slices, to different cocaine concentrations. One possible mechanism mediating these effects is changes in DA autoreceptor sensitivity. Daily, intermittent cocaine injections may result in DA autoreceptor desensitization, while the continuous infusion of cocaine may result in DA autoreceptor supersensitivity. The present experiment examined this possibility. The animals were pretreated with 40 mg/kg/day of cocaine for 14 days by either subcutaneous injections or continuous infusion by osmotic minipumps. The rats were then withdrawn from the treatment regimen for 5 days. After 5 days, the rats were sacrificed, and caudate-putamen slices quickly obtained. The slices were then placed in glass perfusion chamber, and superfused with artificial CSF for 60 minutes. At the end of this period, the slices were electrically stimulated with a train of supramaximal, unipolar, rectangular waves (20 ma, 2 msec) at 3 Hz (50 pulses). Ten 2 min samples were collected. This electrical stimulation was given 60 minutes later (S2). However, 30 min prior to the S2 period, the brain slices were perfused with either 0.1 μM DA or an extracellular dopamine antagonist. The data were analyzed by determining the [S2/S1] ratio for different superfide concentrations. This research was supported by NIDA grant DA06360, and NIH grant T32: MH51717.
450.15
DOSE-DEPENDENT CHANGES IN THE RAT DOPAMINERGIC RECEPTOR SYSTEM AFTER CHRONIC ADMINISTRATION OF COCAINE. W.W. Albrecht*†, H. Narang, C. Johanson*, & T. Psychiatry Research Institute, Fargo, ND 58103; †Medical School of Zulia, Maracaibo, Venezuela.

We have previously reported time-dependent alterations in the rat dopaminergic receptor system after fixed doses of cocaine (15.0 mg/kg, i.p.). In the present study, dose-dependent effects of cocaine on the dopaminergic system were determined. Rats were injected with cocaine (5.0, 10.0, 15.0, 20.0, and 25.0 mg/kg, i.p., b.i.d.) or saline for a 21 day period. [3H]Cocaine and [3H]SN23983 binding in striatum and cortex from animals injected with 10.0 and 15.0 mg/kg were significantly higher than the control animals. [3H]DPFC binding in striatal tissue was significantly increased in animals injected with 10.0 and 15.0 mg/kg of cocaine; however, in cortices of these animals, significant changes were seen with only 15.0 mg/kg of cocaine. Changes in [3H]halocaine binding in these tissues were reversed by time. These results indicate that chronic exposure to cocaine produces a dose-dependent upregulation in cortical and striatal D1 and D2-uptake sites.

450.17

It is well established that intermittent schedules of psychostimulants produce behavioral sensitization while continuous regimens tend to cause tolerance. In-vivo microdialysis was utilized to examine extracellular striatal dopamine and monoamine metabolite (DOPAC, HVA, 5-HIAA) levels at different time periods in cocaine and non-cocaine animals. Male Sprague-Dawley rats were implanted with subcutaneous cocaine pellets which release approximately 105mg of cocaine freebase over 5 days. Rats were sampled before the beginning cocaine administration, on day 5 of cocaine, 24 hours post cocaine or 7 days post cocaine. Time-response relationships were established by infusion of quinpirole (QP) through the dialysis probe (10^-7, 10^-6, 10^-5 M). DA levels decreased in dose response fashion with no group differences among animals on day 5. Time-dependent changes were observed in the striatum or amygdala. These structures were perfused (0.5 uL/min) with 4mm probes during haloperidol induced DA release (1 mg/kg, I.P., n = 8). The dose-dependent upregulation in cortical and striatal D1 and D2-uptake sites.

450.19
QUANTITATIVE MICRODIALYSIS UNDER TRANSIENT CONDITIONS. B.J. Olsen* and J.B. Justice Jr., Dept. of Chemistry, Atlanta, GA 30332.

A method based on the point of no net flux (Liangard et al., Am. J. Physiol. 256:E250-E255, 1988) has been developed for quantitative microdialysis under transient conditions. The method uses a relationship between the number of pulses per period of 10-20 or 40 min dopamine (DA) at 6.6 ml/min. Dialysate was collected and measured for time intervals prior to and during drug administration. Data from the four groups were divided by their respective DA time course and used to determine the in vivo recovery and the extracellular concentration of DA as a function of time. It was hypothesized that changes in uptake and release would change probe recovery. No change in recovery was observed in the striatum with 4 probes during haloperidol induced DA release (1 mg/kg, I.P., n = 6 per group). However, it is possible that the already high baseline recovery (78%) masked other evident changes. In order to examine the effect of uptake inhibition on recovery, cocaine (20 mg/kg, I.P., n = 16 per group) was administered and dialysate was collected using 2mm probes in the nucleus accumbens. Recovery decreased from 98% at baseline to 25% twenty minutes following drug administration and returned to 48% seventy minutes following drug administration. A similar recovery was observed in extracellular DA than was evident in dialysate. These results suggest that probe recovery is dependent on active neurotransmitter properties such as uptake and release (Parsons et al., J. Neurosci. Meth., 40:131-137, 1991). The present method appears useful for quantitatively characterizing the pharmacology of neurotransmitter systems.

450.20
THE EFFECTS OF DISCRETE LESIONS OF THE SUB-COMMISSURAL VENTRAL PALLIDUM ON COCAINE SELF-ADMINISTRATION IN THE RAT. J. Hubbell and G.G. Koob. The Yerkes Regional Primate Research Institute, La Jolla, CA 92037.

The involvement of the nucleus accumbens in mediating cocaine reinforcement has been largely established. Previous work from our laboratory has demonstrated that ibotenic acid lesions of one of the output regions or the nucleus accumbens sub-lenticular substantia innominata, produced significant decreases in the highest ratio obtained in rats self-administering (SA) cocaine. In this study, we investigated the importance of another accumbal lesion, the sub-commissural ventral pallidum, in mediating the reinforcement properties of cocaine in the rat. Animals were trained to self-administer cocaine (0.75 mg/kg i/v) via an intravenous catheter on a FR5 schedule of reinforcement. Subsequently, rats were either given bilateral i.p. injections (0.5 ?L per side) of ibotenic acid (10 ?g/ul lesion group) or vehicle (sham group) into the sub-commissural ventral pallidum. Five days post-lesion, cocaine SA on a FR5 schedule was resumed for three days. Next, a dose effect function was determined in one 3 hour session. A progressive ratio schedule in which the ratio requirement was increased after each reinforcement was also used. The lesion group showed a significant decrease in FR5 responding for cocaine five days after the lesion as compared to the sham group. While the lesion produced decreases in responding for cocaine at all doses, the rate of responding was inversely proportional to the dose. However, compared to sham animals, in the progressive ratio task, no effect was found in the total number of rewards or in the highest ratio obtained in lesioned rats. These results, taken together with our previous data, support the hypothesis that the region commonly called the ventral pallidum is a heterogeneous structure which may have an equally heterogeneous relationship with the nucleus accumbens. Further, these results suggest that within the ventral pallidum there are some areas that are more critically involved in cocaine reinforcement than others. Supported by NIDA grant DA04398.

Chronic administration of MPTP to adult marmosets induces alterations in brain monoamines, serotonergic, and peptide systems, but it is not known if MPTP can also produce neurotoxic effects on the developing brain. To study the possibility of MPTP to produce neurotoxic effects, we administered to two female marmosets who became pregnant. The monkeys received six injections, twice a week, during the whole gestational period, except for the last 15 days before term, when pregnancy was noticed. Baby marmosets (n=3) were sacrificed 3 months after birth and levels of dopaminergic (DA), 5-hydroxytryptaminergic (5-HT) and its metabolites, DopAC, HVA, 5-HIAA were determined in several brain regions compared to age-matched controls (n=6). Substance P (SP) content was measured in the basal ganglia by RIA. Significant reduction of DA and its metabolites, DopAC and HVA, was found in the caudate and putamen and n. accumbens of marmosets exposed “in utero” to MPTP, but not in other areas studied. In contrast to the extensive and severe 5-HT loss induced by chronic MPTP treatment of adult animals, no change in striatal 5-HT content was found in baby marmosets and only extrastriatal 5-HT systems were affected. A significant decrease in SP levels was observed in the n. nigra of baby marmosets. The results demonstrate that MPTP can cross the placenta and exert its neurotoxic effect on monoamine and peptide systems in the fetal brain of primates. These findings show the possibility of “in utero” lesion of several neurotransmitter systems in the marmoset brain and indicate that non-methylated neurons (as it is the case in the fetus) are also susceptible to MPTP. (Supported by EEC and JALS Fdn.)


The short term symptomatic improvement in Parkinson’s patients with Sinemet can be very dramatic. However, progressive increase in disability of some patients coupled with toxic side effects from toxic oxidation of L-dopa and dopamine suggested the possibility of L-dopa causing further deterioration of the disease. We tested the effects L-dopa and Sinemet on 6-OHDA induced implanted rats. L-dopa (344 mg/kg) was given 1 b.i.d. (50mg L-dopa equivalent/kg) for eight weeks into 12 Fisher 344 rats whose nigrostriatal system was partially lesioned with unilateral intra-nigral injection of 4 μL 6-hydroxydopamine. Substantia nigra demonstrated rotund response to amphetamine (5mg/kg), but not to amphetamine (0.25mg/kg). Saline injections were given b.i.d. to 11 control rats matched for the amphetamine induced rotation. The L-dopa group showed 38% reduction in amphetamine-induced rotation, whereas the control group showed no change from the baseline (p<0.05). The animals in either the total high affinity DA uptake sites or D1-receptors as measured by GBR and SCH23390 autoradiography. Spiperone D2-receptor showed a decreasing trend in the L-dopa treated group (p<0.05). The trunical and limb dystonia following L-dopa injection were also measured. No further progression of dystonia was observed throughout the duration of the experiment. Our results thus far suggest that this time course of L-dopa treatment does not appear to cause further decline in the nigrostriatal dopamine system in partially lesioned rats. Supported by grants from the UPF & NIH NS24032.

451.5 AKINESIA DURING DOPAMINE AGONIST INDUCED CIRCLING BEHAVIOR AFTER SEVERE UNILATERAL NEOBROSTRAL DOPAMINE DEPLETION IN RATS. D. Norton, T. Schallert*, and T.A. Jones. Neuroscience Inst and Dept. of Psychology, Univ. of Texas, Austin, TX 78712.

Circling behavior after unilateral 6-hydroxydopamine (6-OHDA)-induced neuronal dopamine (DA) depletion has been widely used as a model for screening anti-parkinsonian agents. However, the unilateral 6-OHDA model, as presently used, does not mirror one of the most debilitating symptoms of Parkinson’s disease: akinesia. During circling, all limbs make stepping movements, including the limbs contralateral to the lesioned hemisphere. Hence, this study used a novel behavioral test was used to isolate and compare stepping movements of the ipsilateral and contralateral limbs of rats with unilateral nigrostriatal DA depletion. We found that in the undrugged condition and under amphetamine, the ipsilateral (non-impaired) forelimb shifts the weight of the animal to a new location in space (DA-dependent stepping), whereas the contralateral forelimb makes only catch-up steps designed to re-establish support of the body weight (DA-independent stepping). When the contralateral forelimb is isolated so that it alone bears the weight of the animal, the hindquarters show typical stepping (ipsilateral and contralateral limbs off the ground), it is akinetic. Preliminary results indicate that when the depletion is severe, the DA agonist apomorphine causes contralateral circling in the ipsilateral limb, but does not reverse akinesia in the contralateral forelimb. The role of DA in movement initiation will be discussed in view of these data. (Supported by NS 23964.)


The study of moderate dopamine (DA) lesions in animals could serve as a model of the preclinical stage of Parkinson’s disease, both in the search for possible beneficial therapies and in understanding disease progression. Therefore, we studied asymmetries in turning and thigmotactic scanning in rats with unilateral injections of 6-OHDA into the substantia nigra and compared them with the degree of striatal DA depletion. Animals with severe lesions (<20% residual DA) showed spontaneous ipsiversive turning and more scanning with the side of the face ipsilateral to the lesion. These asymmetries were reversed by apomorphine. Animals with moderate depletions (20-65% DA) did not show spontaneous asymmetries in turning, but an ipsilateral asymmetry in scanning. Most important is that out of this group, animals with 45-65% residual DA levels showed ipsiversive turning and ipsilateral scanning under apomorphine. These results suggest that scanning is a more sensitive measure than turning for the detection of functional asymmetries after moderate striatal DA lesions. The ipsiversive asymmetries observed after apomorphine in animals with moderate depletions might be due to ipsilateral changes in self-regulatory mechanisms of the nigrostriatal DA system, such as in DA autoreceptors, which in turn might be related to compensatory mechanisms in the preclinical stage of Parkinson’s disease.

451.4 SUBTHALAMIC NUCLEUS ABLATION AS A METHOD FOR REDUCING APOMORPHINE-INDUCED ROTATIONAL BEHAVIOR IN 6-OHDA LESIONED RODENTS. B.H. Hallas*, R.M. Cebelanski, P. Jacobina, M.F. Zajankala, E. Fatemi. N.Y. College of Osteopathic Medicine, N.Y. N.Y. University, N.Y.

A rodent model for Parkinson’s Disease is the chemical lesion of the Substantia Nigra (SN) with 6-hydroxydopamine (6-OHDA). We studied the effects of following apomorphine administration, results in quantifiable rotational behavior. This study investigated the influence of the Subthalamic Nucleus (STN) on ipsilateral rotational behavior with 6-OHDA and induced to rotate with apomorphine. Adult rats received stereotaxic injections of 6-OHDA into the left SN. After 21 days, apomorphine was injected intra-peritoneally once a day for 14 additional days. Ten minutes after the injection, the number of rotations were counted for each animal over a period of 15 minutes. After establishment of the rotational baseline (at 14 days), the left STN was ablated by stereotaxic electrolytic lesions. Control animals received no STN ablation. All animals were then returned for rotational behavior as above after a recovery period of 14 days. It was found that the majority of the experimental animals demonstrated a significant reduction of rotational behavior compared to controls. However, four animals exhibited ipsilateral rotation following STN ablation (i.e. to the left). These animals were tested for 14 additional days and then received an electrolytic lesion to the contralateral (right) STN. After a second operation time these animals were retested for rotational behavior following apomorphine injection. It was found that these animals then demonstrated a significant decrease in rotational behavior. It can therefore be concluded that ablation of the STN ameliorates apomorphine-induced rotational behavior following 6-OHDA SN lesions possibly by reducing abnormally increased medial globus pallidus output.


In rodent and primate models of Parkinson’s disease, antagonists of the NMDA and AMPA subtypes of glutamate receptors have been shown to synergize with L-DOPA to reverse parkinsonian motor defects. In this study, we have used monoaminergic depleted rats to examine the antiparkinsonian potential of remacemide (Fisons), an anticonvulsant with activity at the NMDA receptor ion channel. Male Sprague-Dawley rats (125-150 g) were rendered akinetic by administration of reserpine (5 mg/kg, i.p.) 24 h prior to testing. Motor activity was quantified using cages equipped with infrared sensor beams in reserptized rats. L-DOPA increased horizontal locomotor activity, while substantial co-administration with remacemide (5-40 mg/kg, p.o.) there was a dose-dependent increase in horizontal locomotor activity unlike MX-801, remacemide does not produce locomotor stimulatory effects. Moreover, remacemide potentiates the effects of L-DOPA over a broad dose range, in contrast to MK-801 and CPP. (This work was supported, in part, by the Hope Geoghegan Fund and the Davenport-Hatch Foundation.)

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The extent and status of a unilateral lesion of the nigrostriatal dopamine (DA) system has typically been assessed by quantifying rotational asymmetry in response to DA agonist administration. Since agonists-induced, whose mechanism is still obscure, may not be a good behavioral index of parkinsonism, particularly in monkeys and humans, we examined the extent to which the status of volitional motor function, perhaps a better index of parkinsonism in monkeys, and rotational asymmetry coincide. In particular, we examined whether rotational asymmetry, a metabolically simple behavioral index, could accurately reflect the functional status of hemi-parkinsonian monkeys. Four male rhesus monkeys (approx. 4 yrs. old) trained to perform a series of tasks under both base line and naloxone conditions, were trained to show a consistent contralateral rotation to a specific task, a condition of a double simulation, and lateralized attention (i.e., response to double simultaneous stimulation, impos to lateralized moving stimuli, and lateralized reward retrieval). Animals were then made hemi-parkinsonian by intracarotid injection of MPTP (25 mg/kg) 2 and re-tested on the above mentioned tasks over the next year. Within the first 6 months after lesioning, animals showed dose-dependent rotational asymmetry is apomorphine or DA agonist N0923 stimulation and significant unilateral impairment in motor function (with the use of the limb contralateral to their lesion) and amnesic abilities (assessed with the limb ipsilateral to their lesion). Six to 12 months after lesioning, 3 animals showed significant improvement in performance of volitional motor tasks and all monkeys showed distinct reversal of at least some of their initial lateralized attention deficits. Despite this behavioral improvement, rotational asymmetry in response to dopamine agonists was still intact and in many cases more than when the animals were behaviourally impaired. These results suggest that over time, functional improvement can occur in hemi-parkinsonian monkeys and that rotational asymmetry may not be a good index of the functional status of the substantia nigra dopamine system in the monkey. Supported by Whitby Research, Inc. and NIH 46531.


GM1 has been reported to protect pigmented neurons of substantia nigra from MPTT lesion. We evaluated behavioral effects of GM1 treatment in 3 Cebus apella with a persistent hemiparkinsonian syndrome (Pathology). In lieu of GM1 (5mg/kg) p.o. daily 75 days while control monkeys (n=22 months of an intracarotid infusion of MPTP-HCl (1.2mg/kg)). MPTT treated and normal monkeys (2-4kg, 13-16 years) were trained in a primate chair to solve motor-cognitive tests. Normal animals covered in 2-3 sessions the 3 levels, while manual preferred between tests MPTT monkeys could not resolve level 2 or 3. The hemiparkinsonian monkeys received 20 daily saline injections or GMFI (5mg/kg) daily for 28 days, ongoing evaluated as (CB-9) in a primate chair weekly. After 3 weeks of saline or GM1, circling activity was tested before and after a dopamine administration (0.4mg/kg). During saline or GM1 treatment the animals did not improve their performance and the apc tests did not reveal changes in circling behavior. At present time we are conducting a follow up GM1 treatment. Supported by: CONICET, CERES, CEMIC, Petrolera Argentina S Jorge.


U-91356A is a novel dopamine agonist, (5S)/(propylamino)-5,6-dihydrop-4(1H)-imidazol-4(5,1-q)-quinolin-2(1H)-one hydrochloride. In reserpina induced NJSA (Harlan CF 1 mice, U-91356A caused locomotor stimulation at 10 and 10 mg/kg p. Apomorphine HCl (apo) 6.5, 10 mg/kg), abate H-S (basal, (q)-quinoline HCl (q), and pergolide meylate (per) were also stimulated in reserpina mice, but bromocriptine meylate (brom) was not. In the same reserpina mice, U-91356A and its antagonisted apo-induced locomotor stimulation; bromo was slightly active while apo and per were inactive as antagonists. In normal, nonreserpina B6C3F1 mice (Harlan) all of the above compounds antagonized amphetamine. U-91356A was active orally and i.p. as well as p. i. In Sprague-Dawley (Charles River) rats with unilateral 6-OH dopam onine lesions of the substantia nigra, U-91356A caused centralrotational turning at 0.3 mg/kg and saline (0-10) and at 8 days post-treatment tested in a habituation-dishabitation paradigm. Two-3 minute presentations of the same stimulus (ovarclometrics) female (habituation) was followed by three-2 minute presentations using a different stimulus female (dishabituation) with an inter trial interval of 20 minutes. Investigation lines (± SEM in seconds) of saline animals decreased over the habituation trials (103 ± 10, 63 ± 10, 37 ± 9) while those of MPTT animals did not decrease until the third trial (95 ± 11, 85 ± 12, 43 ± 4). A clear dishabituation response from trial 3 to 4 was shown by saline (37 ± 9 to 72 ± 11) but not MPTT (43 ± 4 to 58 ± 9) animals. Treatment of MPTT animals with L-DOPA one hour prior to test (50 mg/kg, p.o) resulted in habituation (94 ± 8, 45 ± 8, 28 ± 4) and dishabituation (93 ± 10) scores which were similar to saline-treated animals. These results demonstrate that MPTT treatment disrupted initial habituation and dishabituation responses in male mice while treatment of MPTT with L-DOPA restored their responses to that observed in saline animals. Such findings suggest that administration of the dopaminergic neurotoxin MPTT impairs social memory/recognition processes, an effect which can be rectified with exogenous L-DOPA treatment.


U-91356A, (U-91, or (5S)/(propylamino)-5,6-dihydrop-4(1H)-imidazol-4(5,1-q)-quinolin-2(1H)-one hydrochloride) is a novel dopamine (DA) agonist that binds to both D2 (K1 = 1.3 nM) and D17A (K1 = 58 nM) receptors. U-91 did not bind at 1 uM to a variety of biochemical adrenergic, 5HT, cholinergic, DA, benzodiazipe line or opiate receptors. Bromocriptine, lisuride, and pergolide all had high binding affinities for several non-DA receptors. U-91 (ED50 = 22 ug/kg i.v.), bromocriptine (CB50 = 1804 ug/kg), pergolide (ED30 = 23 ug/kg) and lisuride (ED50 = 74 ug/kg) all depressed substantia nigra DA neuron firing, but this was complete only with U-91 cH. Higher U-91 doses depressed dornal raphe 5HT neurous (ED50 = 204 ug/kg). U-91 was orally available in rats (pov 6%) and monkeys (pov 28%). In MPTT-treated monkeys, U-91 successfully reversed parkinsonian symptoms both substanteaely (20 ug/kg) and orally (2 mg/kg), duration 7 hr. The data suggests that U-91's greater selectivity, efficacy and oral availability could provide a novel improved treatment for Parkinson's Disease.

451.12 THE EFFECT OF MPTT ± L-DOPA UPON SOCIAL MEMORY/RECOGNITION IN MICE. D.E. Plisak* and J. Kreuzberg. Department of Anatomy, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Retired breeder male CD-1 mice were treated with either MPTT (10 mg/kg p, cld, xid, N=10) or saline (N=10) and at 8 days post-treatment tested in a habituation-dishabitation paradigm. Three-2 minute presentations of the same stimulus (ovarclometrics) female (habituation) was followed by three-2 minute presentations using a different stimulus female (dishabituation) with an inter trial interval of 20 minutes. Investigation lines (± SEM in seconds) of saline animals decreased over the habituation trials (103 ± 10, 63 ± 10, 37 ± 9) while those of MPTT animals did not decrease until the third trial (95 ± 11, 85 ± 12, 43 ± 4). A clear dishabituation response from trial 3 to 4 was shown by saline (37 ± 9 to 72 ± 11) but not MPTT (43 ± 4 to 58 ± 9) animals. Treatment of MPTT animals with L-DOPA one hour prior to test (50 mg/kg, p.o) resulted in habituation (94 ± 8, 45 ± 8, 28 ± 4) and dishabituation (93 ± 10) scores which were similar to saline-treated animals. These results demonstrate that MPTT treatment disrupted initial habituation and dishabituation responses in male mice while treatment of MPTT with L-DOPA restored their responses to that observed in saline animals. Such findings suggest that administration of the dopaminergic neurotoxin MPTT impairs social memory/recognition processes, an effect which can be rectified with exogenous L-DOPA treatment.

A new method assessing the forelimb reaching capabilities of individual footpaws in the rat has recently been described (Montoya et al., J. Neurosci. Meth. 36:219-228, 1991). Using this test, Montoya et al. (1991) report that 6-OHDA-induced deficits that were reported by Montoya et al. who use 2 pellets/well. The present study was designed to determine whether the amount of food provided during training is a significant factor in reaching training. Two groups of normal rats (n=10) were trained for 5 consecutive days (15 min/day) with either 10 pellets or 2 pellets/well (80 total pellets vs. 16 total pellets) and then the conditions were crossed-over for training on days 6-10. Prior to training, both groups were reduced to <85% of pre-training body weight and maintained at this level by supplemental feeding when necessary. Rats given the opportunity to eat 80 pellets per session learned well and continued to improve performance when switched to 2 pellets/well, eventually consuming 75% of the pellets available. In contrast, the rats initially trained with 2 pellets/well are few pellets and never consumed more than 5% of the pellets even though they had access to 80 pellets on days 6-10. In addition, the group which originally had access to 80 pellets was able to maintain >85% body weight with little supplementation on days 1-5. This was not the case for the rats trained with 2 pellets/well; they received constant dietary supplements in order to remain above 85% body weight. Thus, the rats initially trained with access to 80 pellets could work to maintain their body weights with a third group condition. Thus, it appears that the a priori motivational level of rats affects the degree of post-6-OHDA lesion deficit as indicated by the different levels of post-lesion performance reported by Gray et al., as compared with Montoya et al. (1990).


Parkinson’s disease (PD) is characterized by a loss of dopaminergic (DA) neurons in the nigrostriatal pathway. PD patients also have decreased retinal DA levels (Javoy and Dufour, 1990), which may interfere with early visual processing. Consistent with this idea, the pattern electroretinogram and spatial and temporal contrast sensitivity are abnormal in PD. In order to explore the generality of these findings, we administered a series of visual psychophysical tests to 10 nondemented PD patients (mean age: 64.9 years and 29 age-matched control subjects (mean age: 67.8 years) upon the Hoehn and Yahr stages of disability, 1 patient was classified as Stage I, 8 as Stage II, and 1 as Stage III. All PD patients were receiving DA medications. Psychophysical measurements were made of fine pattern discrimination, color discrimination along the red-green and blue-red primary color axes, and contrast sensitivity to flickering (0.75, and 15 Hz) and moving (3.15, 4.72, and 9.44 )/s) stimuli. In all cases, stimuli were presented within 1.0 x 1.0 degree of retinal eccentricity of 75°. Subjects responded in a four-position forced-choice procedure, and thresholds were determined with the method of constant stimuli. Under these conditions, the performance of PD patients did not differ significantly from age-matched control subjects on any of the visual capacities tested. It appears that some visual capacities are spared in PD, at least in patients who are taking DA medication.


The human skeletal muscle sodium channel gene (SCNA) has been linked to neuromuscular disorders. Since there are at least 16 different sodium channel genes that have been identified, it is possible that different sodium channel abnormalities may be present in different muscle disorders. To determine if the sodium channel gene is involved in all neuromuscular disorders, we have isolated and sequenced the entire sodium channel gene from different neuromuscular disorders. The results show that the sodium channel gene is highly conserved between different human species.

452.2 SEVERE ADULT-ONSET NEUROMUSCULAR DISORDER IN A TRANSGENIC LINE OF MICE. B. Popko1, D. Kelly2, A. Milatovich3, U. Franko4, and K. Suzuki5. 1Dept. of Pathology, Brain and Development Research Center, U. of North Carolina, Chapel Hill, NC 27599. 2Dept. of Genetics and 3Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305

We have generated a transgenic line of mice in which the transgene has disrupted the function of an endogenous gene essential for normal muscle function. Mice homozygous for the BFPD transgene in our transgenic line (BFPD1) develop severe neuromuscular disease. This transgene is not expressed in normal muscle, but is expressed in skin and muscle of mice with severe neuromuscular disease. The muscle phenotype includes muscle weakness and wasting, and the skin phenotype includes hyperkeratosis and abnormal hair shafts. The transgene is expressed in muscle, and the muscle phenotype is rescued by disruption of the transgene. The muscle phenotype includes muscle weakness and wasting, and the skin phenotype includes hyperkeratosis and abnormal hair shafts. The transgene is expressed in muscle, and the muscle phenotype is rescued by disruption of the transgene. The muscle phenotype includes muscle weakness and wasting, and the skin phenotype includes hyperkeratosis and abnormal hair shafts. The transgene is expressed in muscle, and the muscle phenotype is rescued by disruption of the transgene.

NEUROMUSCULAR DISEASES I
TERMINAL MOTOR AXON MORPHOLOGY IN HOMOZYGOTOUS HEREDITARY CANINE SPINAL MUSCULAR ATROPHY.
K. Alderson and L.C. Cork
Department of Neurology, University of Utah, Salt Lake City, Utah and Division of Comparative Medicine, Johns Hopkins University, Baltimore, Maryland

Dogs homozygous for hereditary canine spinal muscular atrophy develop rapid progressive muscle weakness. We evaluated the morphology and myelination of distal motor nerves in the tail base perineural muscle of homozygous pups at three stages, early, middle, and late, to determine if muscle weakness correlated with abnormalities of distal motor axons. Compared to age-matched normal dogs, early affected pups had increased unmystylinen axonal collaterals and increased terminal innervation ratio. Mid-stage pups had thin, unmyelinated axonal collaterals, and evidence of Wallerian degeneration in intramuscular nerves. End stage pups (9 weeks of age) had a paucity of motor axons, an irregular distribution of neurofilaments within the axon, and aberrant growth cones. We conclude that as hereditary canine spinal muscular atrophy progresses, intramuscular axon motor axons are lost, thin unmyelinated axonal collaterals develop, and the organization of neurofilaments is altered. Both the loss of motor axons and the lack of myelin may have clinical and electrophysiological consequences.

ALTERNATIONS IN NEUROFILAMENT mRNAs IN HEREDITARY CANINE SPINAL MUSCULAR ATROPHY. N.A. Muma and L.C. Cork
The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

Hereditary canine spinal muscular atrophy (HCSMA) is a dominantly inherited motor neuron disease in which distal axonal caliber is reduced in lower motor neurons. Because several animal models show that neurofilament protein gene expression is a major determinant of axonal caliber, we examined neurofilament gene expression in HCSMA early in the clinical disease. Using quantitative in situ hybridization we found that the levels of mRNA encoding the low molecular weight neurofilament protein subunit (NF-L) were significantly different from levels of mRNA encoding the high molecular weight neurofilament protein subunit (NF-M) and poly-A mRNA in cervical spinal cord enlargement of dogs with HCSMA compared to control dogs. The levels of poly-A mRNA in neurons in the same regions were comparable in dogs with HCSMA and controls. If neurofilament protein subunit levels are found to follow the mRNA levels in this animal model, our results would suggest that decreased expression of the NF-L gene is sufficient to inhibit neurofilament function, i.e., maintenance of axonal caliber, particularly by disrupting normal neurofilament assembly.

DYSTROPHIN DISTRIBUTION IN MUSCLE AND NEURONS OF DUCHENNE MUSCULAR DYSTROPHY HUMAN FETUSES.
Umeharu, S. Tachibana, and S. Graeme
C.N.R. Unit for Muscle Bolt. & Physiopath., Padova; Dept. of Cytomorphol., Med. School, Cagliari (Italy)

Duchenne Muscular Dystrophy (DMD) is the result of the deficiency of a specific cytoskeletal protein called dystrophin, which binds to the inner plasma membrane. We have previously shown that dystrophin can be present in DMD human fetal neurons in culture. Therefore, we analyzed the distribution of dystrophin and spectrin in muscle and neurons of two DMD human fetuses (18-14 weeks) with immunocytochemistry. Different muscles were frozen and serial sections (10 µm thick) were incubated with monoclonal antibodies against spectrin, the cytoskeletal portion of dystrophin and different isoforms of myosin heavy chains (MHC). Neurons were grown in culture as previously described. Immunochemistry showed that all myocytes and the low immaturity spectrin were positive for β-spectrin, but negative for dystrophin. On the contrary, some neurons were positive for dystrophin and all showed spectrin staining. This suggests the presence of adult slow MCH in some young myocytes and in myotubes. These results help in understanding the mechanism of expression of different genes, such as the DMD gene, during human development. (Supported by Telethon-Italy, 1991).

MOTOR UNIT PROPERTIES IN HEREDITARY CANINE SPINAL MUSCULAR ATROPHY. M.J. Pitts, L.C. Cork, and N. Wallace
Department of Anatomy and Neurobiology, Medical College of Pennsylvania and Div. of Comparative Medicine, Johns Hopkins University School of Medicine.

Hereditary canine spinal muscular atrophy (HCSMA) is an autosomal dominant disease which is characterized by progressive weakness and eventual loss of lower motor neurons. Using intracellular recording and stimulation of single motor units, we have studied medial gastrocnemius (MG) motor unit properties in a litter of HCSMA pups aged 10-13 weeks. In units investigated thus far, motor units were found to respond normally to low frequency (1 Hz) antidromic stimulation of peripheral axons and to possess electrical properties similar to adult cat motor neurons. In heterogeneous pups (4), the motor unit population was composed of types FR and S units with no evidence for the existence of type FF units. We have not yet confirmed this motor unit composition in normal animals, but the predominance of types FR and S units is consistent with available enzyme histochemistry evidence for normal adult canine MG muscle. In 2 homozygous pups studied thusfar, two populations of units were detected. In the first, intracellular stimulation of motorneurons failed to produce detectable motor unit force despite the presence of antidromic motorneuron activation following peripheral MG nerve stimulation. This failure occurred in 30-50% of the tested motor neuron population and was associated with an inability to detect EMG responses linked to the intracellular stimulation. In the other population, stimulation produced measurable motor unit mechanical responses best characterized as types FR and S but with force outputs lower than in the heterozygous group.

LOCALIZATION OF DYSTROPHIN IN HUMAN AND MONKEY BRAIN. Johny Huard, Pierre-Yves Côté, Jean-Pierre Bouchard, Carol L. Richards and Jacques P. Tremblay
Neurobiology Laboratory, Enfant-Jésus Hospital, 1401, 18th Street, Quebec (QC), G1J 1Z9

Duchenne muscular dystrophy (DMD) has been characterized by a lack of dystrophin expression. This protein (420 kDa) is localized in the sarcolemma of normal muscle fibers and is absent of DMD and mdx skeletal muscle. Dystrophin would be involved in maintaining muscle membrane integrity during repeated cycles of contraction and relaxation. Molecular biology techniques have permitted to detect dystrophin transcript in the central nervous system (CNS). In fact 50% of DMD patients suffer also a mental retardation probably due to learning and memory problems. Dystrophin was localized in several brain regions of human and monkey brains using antibodies against the red rod domain of dystrophin. A clear visualisation of dystrophin was permitted by an indirect immunoperoxidase method using an amplification with biotin and avidin. Dystrophin-like immunoreactivity was observed in brain regions involved in learning and memory such as hippocampal formation, cerebral cortex and the dрастogenic nucleus of cerebellum. A positive reaction was also present in brain regions implicated in motor function (spinal cord, cerebellum, thalamus and substantia nigra). The Western blot analysis confirmed the presence of a 420 kDa polyepitide in these brain regions with both ant dystrophin antibodies. However the rod domain antibody also detected a dystrophin-like protein. These results raise the possibility that the lack of dystrophin is involved in the cognitive impairment observed in several DMD patients. However it will be important to analyze more details the clinical symptoms of DMD patient to understand the dystrophin role in brain.
452.9
RESTORATION OF FAST AXONAL TRANSPORT IN MUSCLER MICE. H. Mitsumoto, J.M. Jacob, K. Kurahigali, and J.G. McQuarrie*, Cleveland VA Med. Ctr., and Cleveland Clinic Foundation, Cleveland, OH 44106.

To establish a neuronal function in a mutant mouse model of motor neuron disease, we have examined anterograde axonal transport in hindlimb motor neurons of wobbler mice. For fast transport, tritiated amino acids were injected into the sciatic nerve. Transport distances were determined from the distance of the fluorescent label at 2 or 3 hr. Transport was 18% slower in wobbler mice than in unaffected littermates (p<0.01). Slow transport, [35S]methionine was injected. Transport rates were determined from the peaks of labeling for structural proteins separated by SDS-PAGE after 2 or 3 hrs. Rates for slow components (SCa) and b (SCb) were unchanged by wobbler disease. Because the rate of fast retrograde transport is also unchanged, we conclude that the slow axonal transport rate is a genetic abnormality in wobbler mice. Therefore, we conclude that slow axonal transport may be accompanied by the neuronalopathy is unlikely to affect the fast transport rate, we suggest that an axonopathy may be affecting the fast transport motor (kinesin).

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452.11
Excitatory Amino Acid Receptors in the Cervical Spinal Cord of the Wobbler Mouse: A Quantitative Autoradiographic Analysis. C. Krueger*, R. Li, H. Mitsumoto and C. Shaw, Depart. of Medicine and Ophthalmology, University of British Columbia, Vancouver, B.C. and Cleveland Clinic Foundation, Cleveland, OH.

Previous studies have suggested that motor neuron death might develop as a consequence of an excitatory neurotoxic compound with agonist action at certain excitatory amino acid (EAA) receptor subtypes on motor neurons. To investigate the possibility that a selective reduction of EAA receptor subtypes might be observed as a result of motor neuron death subsequent to EAA receptor activation, we measured the distribution and density of EAA receptors in the cervical spinal cord of the wobbler mouse using quantitative receptor autoradiography. Homozygous wobbler mice develop progressive motor neuron loss, especially in the cervical spinal cord and brain stem. NMDDA receptor binding sites (25 and 28 kDa) were located in both the ventral and dorsal horns and displayed little regional variation in binding. AMPA binding sites, evaluated with 20mM[3H]CNQX, were concentrated in the dorsal horn of the rat. Kainate binding sites were labelled with 30 mM[3H]kainate, and receptor binding was located largely in the dorsal horn. [3H]Glutamate binding sites (35mM) were most abundant in the dorsal horn of the spinal cord. NMDDA, kainate, glutamate and AMPA receptor binding densities were not significantly different in the ventral horns of wobbler mice compared to those of control litter mates. These results suggest that motor neuron loss in the wobbler mice is not mediated by excitotoxins acting on EAA receptors.

452.12

Distal axonal degeneration (dying back) is the most common pattern of axonal pathology in most mononeuropathies. We examined the rate of fast axonal transport (FAA) in AC neuropathy, a prototypical model of progressive axonal degeneration. Six-week-old male rats given a single injection of AC (500 mg/kg, i.p.) 20 minutes before or after injection of 3H-hyaluronic acid demonstrated no alteration in the maximal transport rate (front of the curve) for radiolabeled proteins, whereas the glycprotein FACE rate was reduced (by 18%) compared to age-matched controls; data was normalized to the radioactivity present in the DRG. A defect in FAA of fetal glycoproteins was noted up to 49 days following a single 100 mg/kg dose, but was reversible (beginning as early as 7 days) following lower dose (50 mg/kg). Repeated injections of AC (30 mg/kg/day, for 7 days) did not decrease the rate of glycoprotein transport impairment. No alteration in the amount of transported materials was found. A correlation was observed between the degree of FAT deficit and axonal degeneration; following pretreatment with β-amidopropinothio protein which exacerbates axonal loss by AC, AC (75 mg/kg) elicited a marked impairment in both protein and glycoprotein FAT rate. We suggest that repeated, low dose AC exposure leads to an irreversible impairment in the delivery of FAT glycoproteins to the distal axon resulting, over time, in axonal degeneration. Supported by NS19611.

452.13
A STUDY OF THE ADVERSE EFFECTS OF 5-HT PRECURSOR-2

A comparison was made of the adverse effects of seven drug treatments which used 5-HT precursors. The adverse effects were collected from others' publications. The underlying cause of Eosinophilic Myalgia Syndrome (EMS) was sought. Neurologic Hilar-Gangion Syndrome (NHS) was added to these clusters for comparison between EMS and NHS. All clusters were subclassified into eight signs: general, skin, muscle, visceral, neurological, psychiatric, blood physiological, and chemical analysis. The appearances of these signs were expressed numerically, and histogram were drawn to compare the pattern. The similarity of those patterns resulted in two separate group: 1. TRP (tryptophan) group; (TRP alone treatment, TRP plus Naomicin Oxydase, TRP plus tricyclic antidepressant). 2. 5-HTP (5-hydroxy tryptophan) group; (5-HTP alone treatment, and TRP plus co-enzyme). The TRP alone treatment's effects showed little resemblance of pattern to that of EMS. In conclusion, EMS may be caused by combined influences involving by the TRP toxicity with conditions of genetic background and/or underlying metabolic abnormality as the predisposition.

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454 SYMPOSIUM: THALAMIC MECHANISMS OF NOCICEPTION.
F.A. Lent*, Johns Hopkins (Chairman); E.G. Jones, UC Irvine; A.V. Asgarli, SUNY Syracuse, M. Bushnell, Univ. Montreal

The principal sensory nucleus and adjacent nuclei of the thalamus comprise a region that is involved in pain-signalling pathways. Evidence will be presented that there are two separate anatomical domains within this region (and matrix domains), that the spinothalamic tract (STT) terminates selectively within the matrix domain. Inputs from STT may explain the activity of neurons in this region which respond to both innocuous and noxious stimuli (WDR neurons). These inputs contribute to the experience of pain, as well as innocuous thermal sensations and noxious pain.

Following injuries to the nervous system there is evidence of anatomical reorganization of the med and matrix domains. Patients with pain following nervous system injury exhibit physiologic reorganization and increased calcium spike associated bursting activity in the region. These changes in neuronal activity may be related to NMDA associated peptides that are activated by noxious stimuli. The convergence of innocuous and noxious inputs in the region may be related to the pain evoked by innocuous stimuli which occurs in many patients following nervous system injury. Therefore, synaptic and intrinsic cellular mechanisms governing thalamic neuronal activity may mediate acute somatic and visceral pain as well as symptoms of pain syndromes observed following injuries to the nervous system.
456.5  OVARIAN STEROID HORMONES REGULATE GLUTAMIC ACID DECARBOXYLASE (GAD) MESSAGING RNA LEVELS IN THE HIPPOCAMPUS OF THE RAT. N. G. Walland, Laboratory for Neuroendocrinology, Rockefeller University, New York, NY 10021. Estradiol and estrogens influence learning and episodic seizure activity, functions mediated in part by the hippocampus. Normal hippocampal function is dependent on precise interactions between the excitatory glutamatergic and inhibitory GABAergic systems. To determine whether or not ovarian steroid hormones interact with GABAergic neurons of the hippocampus, the levels of mRNA for GAD, the rate limiting enzyme for GABA synthesis, were measured using in situ hybridization histochemistry with 35S-labeled antisense or sense (control) riboprobes transcribed from cDNA sequences provided by Dr. J. Tobin. The levels of mRNA for GAD were analyzed in selected regions of dorsal hippocampus and medial basal hypothalamus in three groups of rats: 1) 2-week-estrous, 2) 2-OX and 2 days of estradiol (E) and 3) E plus 6 h of progesterone (P). Rats were killed at 1500 h. GAD mRNA levels in E rats increased in the GABAergic neurons associated with the pyramidal cell layer in CA1 but not in any other region of the hippocampus. EP reversed the E-induced increase in GAD mRNA in CA1 and induced a small decrease in GAD mRNA in the hilus. No effect of E or EP on GAD mRNA levels was observed at this time of day in any region of these rats measured. In conclusion, estradiol and progesterone may alter learning and seizure activity by altering the function of GABA neurons in the hippocampus.

456.7  LOCALIZATION OF ANDROGEN AND ESTROGEN RECEPTORS WITH GFR AND SRF NEURONS IN RAT HIPPOCAMPUS, HYPOTHALAMUS, THYROID, LIVER, KIDNEY, IN Testes, Brain and Bone Marrow. L. French, W. E. Stumpf, R. A. King, F. M. Wilson and M. Sar. Dept. of Cell Biol. & Anat. Labs. for Reprod. Hlth, Vanderbilt University School of Medicine, Nashville, TN 37232-0150. This study was conducted to determine whether GFR or SRF neurons contain androgen receptor (AR) or estrogen receptor (ER). A dual immunocytochemistry method was applied to visualize AR in GFR and SRF neurons. Adult male rats with or without testosterone propionate treatment (100ng/100 g BW) each received intraventricularly colchicine (50μg/rat), 18-20 h later rats were processed for double immunostaining procedures. Sections were initially stained for AR and ER by the ABC method with antipeptide antibodies AR32 and ER75 respectively using DAB. After receptor staining, the sections were immunostained with GFR and SRF antibodies using 4-chloro-1-naphthol. AR and ER localized in nuclei of neurons of the arcuate, periventricular and ventromedial nucleus where GFR and SRF cell bodies exist. Nuclear localization of AR was found in the majority of SRF neurons but not in GFR neurons. However, ER localization was observed in GFR but not in SRF neurons. These data suggest that estrogen directly affects GRF neurons, while testosterone directly affects SRF neurons. Since testosterone is converted to estradiol in certain brain regions an indirect effect of testosterone on GFR neurons cannot be ruled out. (Supported by NIH Grant 17479 and T32-HD07201.)

456.8  SEXUAL DIMORPHISM OF GALANIN GENE EXPRESSION IN GROWTH-HORMONE-RELEASING HORMONE NEURONS. H.A. Deleninger-van de Wijl, R.A. Brunton, P.B. Kehoe, R.A. Gerber, and D.L. Chipperfield, OhGyn / Physiol Biogy, U. Wash., Seattle, WA 98195. Sexually dimorphic growth hormone (GH) secretion in the rat is likely due, at least in part, to differences in the activity of hypothalamic growth hormone-releasing hormone (GHRH) neurons. Galanin is colocalized in GHRH neurons by immunocytochemistry and stimulates GH secretion. In the current study, we observed that galanin mRNA is colocalized in GHRH neurons and that this coexpression is sexually dimorphic. To test this hypothesis, we performed double-label in situ hybridization on coronal hypothalamic sections obtained from male (n=3) and female (n=5) rats. GHRH mRNA was hybridized with a cRNA probe labeled with digoxigenin-UTP and galanin mRNA was hybridized with a cRNA probe labeled with 35S-UTP. GHRH mRNA-positive cells were visualized by alkaline phosphatase action. Galanin mRNA levels were measured by counting autoradiographic grains over individual GHRH mRNA-positive cells with a computerized image analysis system. We confirmed that GHRH mRNA expression is not different between male and female rats. In addition, we observed a pronounced sexual dimorphism in levels of galanin mRNA in GHRH neurons, with females exhibiting expression 60% greater than males. This sex difference was statistically significant in GHRH neurons of the arcuate (p<0.02) and paraventricular (p<0.001) nuclei and tended toward significance in the ventromedial hypothalamic nucleus (p=0.07). However, in neither sex did GHRH neurons in the paraventricular nucleus express detectable levels of galanin mRNA. We conclude that galanin gene expression by GHRH neurons is sexually dimorphic, and we infer that differential secretion of galanin by GHRH neurons helps to orchestrate the different patterns of the GH secretion between male and female rats.

456.9  THE EXPRESSION OF GALANIN IN LH RH NEURONS IS INHIBITED IN PREGNANT AND LACTATING RATS. J. Merchenthaler*, Functional Morphology Section, NIH, National Institute of Dental Research Triangle Park, NC 27709. Galanin (GAL) coexists with LH RH in a subset of preoptic neurons and functions as a potent LH RH secretagogue. The incidence of colocalization is estrogen-dependent, i.e., the number of cells containing both LH RH and GAL is four-times higher in females. In the present study, a very low incidence of colocalization is reported in pregnant and lactating rats. Cycling female, pregnant and lactating rats were treated with colchicine. Day later, their brains were perfusion-fixed and 30 μm sections were processed. Sections were immunostained for GAL using anti-rat GAL serum and the ABC technique. The presence of uniform GAL-immunoreactive GAL-like neurons, was used as indication for colocalization of the two peptides. While in cycling female rats 75% of the LH RH cells contained GAL in pregnant or lactating rats, the incidence of colocalization was almost undetectable. Progesterone has been shown to decrease the effect of estrogen at the level of the brain and pituitary. The high levels of progesterone present in both pregnant and lactating animals may be responsible for abolishing estradiol-induced increase in GAL expression within LH RH-containing A. In addition, prolactin levels are also high in both animal models; therefore, a role of prolactin in this phenomenon cannot be ruled out. Among other mechanisms, the high levels of prolactin observed during pregnancy and lactation may be responsible for the low incidence of GAL/LH RH colocalization during pregnancy and lactation.

456.10  ESTROGEN INDUCTION OF GALANIN AND IMMEDIATE EARLY GENE EXPRESSION IN THE RAT ANTERIOR PITUITARY GLAND. T. Calcot, K. McDonald*, and L. Koonig*, Dept. of Physiology, Georgetown Univ. Sch. Med., Washington, DC 20007. Estrogen (E) induces galanin gene expression in the rat anterior pituitary gland. A sensitive and specific protection assay was developed to simultaneously quantitate multiple mRNA species in total cellular RNA samples. Time- and dose-dependent effects of E on the expression of c-fos, c-jun, c-myc, and galanin were measured. Each of the indicated genes was expressed in response to E treatment. Galanin mRNA was then upregulated with c-fos mRNA a significant and a strong pattern of c-fos expression. E-stimulated c-fos expression is weakly induced by E, becomes strongly induced by E, and is significantly induced by E. The induction of c-fos gene expression is weakly induced by E, becomes strongly induced by E, and is significantly induced by E. The induction of c-fos gene expression by E is weakly induced by E, becomes strongly induced by E, and is significantly induced by E. The induction of c-fos gene expression by E is weakly induced by E, becomes strongly induced by E, and is significantly induced by E. The induction of c-fos gene expression by E is weakly induced by E, becomes strongly induced by E, and is significantly induced by E. The induction of c-fos gene expression by E is weakly induced by E, becomes strongly induced by E, and is significantly induced by E.
545. 1  
ENDONUCLEASE ACTIVATION IN BRAIN INJURY OF RAT. T. Tominaga*, S. Kura and T. Yoshimoto. Div. of Neurosurgery, Inst. of Brain Diseases and Dept. of Biochem. Genetic, Toho University Sch. of Med., Sendai, Japan 980

The main purpose of this study was to clarify the temporal profile of DNA degradation, especially DNA fragmentation, during the process of brain injury. Additionally, endonuclease activity responsible for DNA breakdown was investigated using its nuclear protein fraction. Adult male Wistar rats were used. Focal cerebral ischemia and freeze-injury were produced by the tandem occlusion of common carotid and proximal middle cerebral arteries, and the application of precooled metal probe to dorsal cortex, respectively, under halothane anesthesia. The DNA was extracted from the lesioned cortex and/or caudoputamen and electrophoresed on a agarose gel. Typical DNA fragmentation into oligonucleosomal sizes was found in the ischemic caudoputamen and the freeze-injured cortex, which amounted to 10% of the DNA around 24 hours after the insult. Coexisting random degradation and specific fragmentation of DNA was observed in the cortex suffered from focal ischemia. To determine whether an endonuclease responsible for DNA fragmentation was present in the brain nuclei, nuclear proteins were extracted from normal brain nuclei and incubated with plasmid DNA or normal brain nuclei under various conditions. This demonstrated that brain nuclei proteins have Ca-dependent endonuclease activity which is related to DNA fragmentation. Moreover, the "gel assay" of endonucleases indicated the NUC1-8, which causes DNA fragmentation in lymphocytes, are also present in brain nuclei.

DNA fragmentation is not unique to programmed cell death and can occur as a result of brain injury, probably through the activation of Ca-dependent endonuclease.

545. 2  

Effect of the competitive AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) antagonist NBQX (6-nitro-7-sulphamoyl-benzo[f]quinoxaline-2,3-dione) was studied in a model of traumatic brain injury in rats. A stainless-steel circular footplate, diameter 4.5 mm, was positioned stereotaxically over the hindbrain area of the motor and sensory cortices of fish. 30 days after injury, rats were sacrificed by transcardial perfusion fixation and the brains were serially sectioned and prepared for immunohistochemistry. Morphological analysis of the brains from 20 vehicle-treated rats (cresyl violet stained sections) showed consistent damage in the neocortex, hippocampal subfields CA3 and CA4 and less severe involvement of the thalamus. Treatment of animals with NBQX (n = 20) led to the reduction of the volume of cortical lesion and a protection against hippocampal damage. These observations suggest that AMPA antagonists may be of benefit for the treatment of acute head injury.

545. 3  

The effect of traumatic injury to the optic nerve on glucose metabolism within the lateral geniculate nucleus (LG) and the superior colliculus (SC) was studied in male Sprague-Dawley rats (N=52, wt=250-300g). Injury to the optic nerve was performed under general anesthesia (33% C2 66% C3, endotracheal intubation). Immediately following the insult, a hole was made in the skull for 1, 5, 30 or 60 seconds. Local cerebral glucose utilization (ICMRglc/μmol/100g/min) was calculated using 14C-labeled deoxy-glucose (250 μCi/pipet) autoradiography at various times following injury. During the 24 post-lesion days, animals were subjected to either ambient light or xenon arc strobe stimulation. Histological examination of the injured rats revealed that the survival duration increased from 12 to 100 days. The CMRGlc in the LG and SC during the injured optic nerve exhibited a pronounced and permanent deficit compared to the contralateral homologous structures, 51±14 % vs 81±9.2, 23.43% (p<0.01) and 58±12.19 vs 83±5.7, 18.73% (p<0.01) in the LG and SC respectively. However, in non-injured animals, particularly those sustaining the 1 sec injury, the CMRGlc depression was restricted to the SC. This metabolic depression spontaneously attenuated within 3 days with animals who sustained the most severe (60 sec) injury. The CMRGlc deficit in the optic nerve animals who had received methylprednisolone (30 mg/kg, ip) every 6 hours began increasing 24 min prior to the insult, the recovery rate from metabolic depression was significantly (p<0.01) different from the uninjured physiological response between the LGN and SC to optic nerve injury, (2) a role for methylprednisolone in the treatment of optic nerve trauma. (NS30008)

545. 4  

Intravesical fibrinogens may be produced by intravesical injection of an agent that is a rational assumption that the entrapping perineum might improve nerve function. Therefore, 1) Establish an experimental model of injection neuraphy; 2) test the effect of toxicity of agents, 0.9% saline and 76% + 1/400 000 epinephrine and 5% phenol, 3) evaluate the effect of perineurones. Methods: Right tibial of the guinea pig (200-300g) is used as the experimental model (N=60). Intravenous injection of three agents is performed by a micropipet and a micromanipulator to guarantee reproducibility. 60 guinea pigs are divided into 4 groups of 15 animals (5 per agent). Grp I: injection only, without treatment. Grp II: injection plus immediate perineurones. Grp III: injection plus perineurones after 1 week. Grp IV: injection plus perineurones after 3 weeks. 32 days after injection, assessment is performed. Assessment parameters: 1) Gastric analysis – A sciatric functional index, adapted to the guat pattern and foot prints of the guinea pig is used. 2) Electrophysiology - Compound action potentials are measured. 3) Histology - Cross sectional biopsies are evaluated by light microscopy and electron microscopy to assess axonal re- and regeneration, myelin changes, response of the perineum to the injury, intrasaccular scarring formation. 4) Quantitative morphometry. Results: Intravesical biopsies show good axonal regeneration and extension of the axonal fibers. These changes are more pronounced after injection of a toxic agent. Phenol produces extensive pathologic changes which include severe peri- and epineurial proliferation, axonal degeneration and endoneurial scarring formation. Injection of maricine and epinephrine produces pathologic changes that range in between saline and phenol injected nerves. Conclusions: Our data show possible beneficial effect of delayed perineurones in nerves injected by a toxic agent.
457.5 HYPERRESPONSE TO PERCUSSIVE TRAUMA IN IMMATURE, MATURE, AND AGED RATS. K. V. Biagas, P. D. Grundy, J. K. Schlichting, and P. M. Kochanek*. Deps. of Anes/Critical Care, Pediatrics, and the Head Injury Research Center, Univ. of Pittsburgh, Pittsburgh, PA 15213. Clinical studies suggest that the cerebrovascular response to head trauma differs with age. Specifically, increased cerebral blood flow, or hyperemia, commonly noted in children is less often seen in older adults after head injury. We hypothesize that reproducible posttraumatic hyperemia can be demonstrated in an animal model and that the extent of hyperemic response differs with age.

Wistar rats, sexually immature (3.5-4.5 wk, N=18), mature (9.15 wk, N=18), and aged (122.14 mo, N=6) were anesthetized and ventilated. Animals exposed to the resected right parietal cortex was produced by weight drop induced to brain weight. Regional cerebral blood flow (rCBF) was determined by 14C-labeled iodoantipyrine in awake normal controls and in rats injured 24 h before. Percent of normal control rCBF was determined for 15 structures. Within group comparisons to controls were made by t-test. At 24 h posttrauma, rCBF was found in all groups in the zone of impact (20% of normal control in immatures, 5% in matures, and 11% in aged rats, all p<0.05 vs. respective normal controls). Hyperemia in the peri-trauma parietal cortex was greatest in immatures (270% of normal control, p<0.05), less in matures (165%, p<0.05), and not found in aged rats (106%, NS). Increased rCBF was found in structures distant to the trauma in all age groups (146 to 188% of normal control in immatures, 66 to 160% in matures, and 157 to 232% in aged). These data indicate enhanced peri-trauma hyperemic response 24 h after brain injury in the immature. However, increased rCBF was found in distant structures in all ages suggesting that the diffuse posttraumatic hyperemic response is not age-dependent in this model.

457.7 MIDDLE LATENCY AUDITORY EVOKED POTENTIALS FOR EVALUATION OF WAKEFULNESS. S. Yasuda*, W. Hayward, L. Duyes, V. Morgese, B. Curtis, and R. Jacobson. Division of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, CA 92356. The medial geniculate body is known to transform tonotopic impulses from brain stem to the hemispheric cognitive function. The authors studied and divided the middle latency (MLP) and brain stem auditory evoked potentials (BAR) at 3-day intervals in 100 patients who sustained head injury and remained and remained awake for certain periods. The results are: 1) Loss and no recovery of BAR wave 5 and MLP indicate severe brain damage (all and Glasgow Coma Scale 3 or 4); 2) Bilateral recovery of BAR and MLP correlates with remaining consciousness including communication capacity; 3) Unilateral recovery of the MLP correlates with return of wakefulness but inadequate communicative capacity; 4) Normal BAR with loss of MLP followed by its complete recovery correlates with rapid return of normal consciousness from continuous sleep.

The authors emphasize the importance of MLP for evaluation and prognostication of wakefulness after head injury.

457.9 CORRELATION OF NEUROFILAMENT IMMUNOHISTOCHEMISTRY WITH SILVER STAINING OF DAMAGED AXONS FOLLOWING HEAD INJURY IN HUMANS. S. Subramaniam Pratimas, and R. D. Rose*, D. J. Graham, J. H. Adams, and T. A. Granelli首 Head Injury Center, Div. of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104, and Dept. of Neuropathology, Subcommittee on Head Injury, General Hospital, Glasgow, Scotland, UK. Diffuse axonal injury in cases of severe head injury is defined by the presence of swollen axonal cytoplasmic and axonal bulbs, defects in reduced silver preparations. The present study sought to establish to what extent neurofilament immunohistomistry correlated with reduced silver staining of damaged axons in the brains of fatal human head injury cases, sub-human primates subjected to experimental inertial head injury, and rats which received directed focrembrain strain injury.

SMI-31, a mouse monoclonal antibody (mAb) to phosphorylated epitope on heavy and medium neurofilament subunits, labels axons in the corpus callosum, subcortical white matter and fasciculi of axons radiating into the cortical gray matter in normal brains. Following head injury in man, baboons, and rats SMI-31 labeled large axonal bulbs in the corpus callosum, subcortical white matter, and deep cortical layers. The density of labeling of the axonal bulbs was identical to that seen in normal sections stained with the Palaner's silver technique. Perikaryas of damaged neurofilamental pyramidal cells in layers III and V were also positively labeled with SMI-31. SMI-32, a postinjury mAb, specifically recognizes heavy and medium neurofilament subunits, selectively labels neurons in layers III and V of normal human, baboon, and rat brains. Following head injury SM1-32 labeled small and medium sized retraction bulbs but not retraction bulbs were among the numerous axons characterized by labeled normal segments continuous with hyperphosphorylated axonal segments disrupted by patches of perikaryal immunoactivity. The authors conclude that SMI-31 and SMI-32 are excellent markers of axonal injury which not only selectively label features of axonal pathology but also identify the perikaryas of damaged axons and may also show light on the cellular mechanisms of axonal pathogy following head injury. Supported by NS-08801-21, NS-28552-02, and the Univ. of Pennsylvania Research Foundation.


Adult male Wistar rats were submitted to a blow of measured intensity to the parietal area of the skull resulting in severe trauma. The mortality of the animals (86%) died within 4-5 days. Some rats received embryonic neocortical grafts and debridement into the lesioned areas immediately after the trauma. These rats exhibited a rapid restoration of their behavioral activities as demonstrated by the open field and acetone test. Intake and motor activity returned to normal on the second post-trauma day. Debridement with no grafts reduced the post-traumatic death rate to 39%. Animals of this group showed reduced activity and food ingestion during the first post-trauma week. Histologic investigation 3 months after trauma and grafting revealed the presence of viable grafts in all hosts. The grafts were consistently well integrated with the host brain. No obvious signs of pathology were observed at the traumatized site of the brain in animals with grafts. The most important results of embryonic neocortex transplantation after severe head injury were mortality reduction, potent stimulation of post-traumatic brain tissue defect repair and the prevention of adhesive processes.


Axonal injury is commonly observed in human traumatic brain injury, but its pathogenesis has remained controversial due to limitations of use of traditional histological methods. Recently, in animals we have shown the utility of monoclonal antibodies to neurofilament subunits, particularly the 68 KD subunit, in detecting damaged axons (Yagami and Pohlhous 1992). In the present study, postmortem analysis was performed on ten human cases at various intervals of postinjury. The monoclonal antibody to the 68 KD subunit was used to identify sites of traumatic axonal injury, which were analyzed at both the light microscopic and electron microscopic levels. This approach revealed at 6 h postinjury axons exhibiting focal swellings. Immunoreactivity was associated with neurofilaments, some of which exhibited clumping and misalignment. Localized infolding of the axolemma was also evident. By 12 h postinjury the continued expansion of sites of focal swelling had progressed in some cases to axonal disconnection. Neurofilaments associated with marked immunoreactivity were now more disordered and vacuolization was observed. At 30 h immunoreactive disconnected reactive swellings displayed convoluted arrays of neurofilaments, partially surrounded by a cap-like zone of electron-lucent lamellae. In advanced stages of survival heterogeneity was evident. These findings reveal that, in head-injured man, axonal disconnection is the result of subtle cytoskeletal change that occurs over a time period. Actual structural/fenctional units undergoing this reactive cytoskeletal change are unknown, but the observed increase in the 68 KD subunit reactivity appears compatible with some local change in phosphorylation status.

Supported by NIH Grant 1932.
458.4 CONTACT WITH MYELIN CAUSES A RELEASE OF CALCIUM FROM INTERNAL STORES IN OLIGODENDROCYTES. S.J. Moorman* and R.I. Hunt. Dept. of Biology, University of Michigan, Ann Arbor, MI 48109.

We are interested in whether interactions between adjacent oligodendrocytes play a role in the process of myelination. As an initial approach, we determined the reaction of neonatal rat oligodendrocytes in culture to an application of crude myelin extract. Myelin extracts were made from either adult rat spinal cord (CNS myelin) or adult rat peripheral nerves (PNS myelin). The extracts used were derived based on their characteristic morphology: some cells with this morphology were confirmed as oligodendrocytes by staining with an antibody to galactocerebroside. We monitored morphology of the leading edge of oligodendrocyte processes using time-lapse video-microscopy, and monitored intracellular free calcium concentrations using fluo-3. Typical experiments were done at 37°C in the normal medium. FURA-2 experiments were done at room temperature in the normal medium. Contact with either CNS myelin or PNS myelin resulted in collapse and retraction of the leading edge of oligodendrocytes. Because collapse of the neuronal growth cone has been associated with increases in [Ca], we tested whether collapse of the oligodendrocyte leading edge was associated with changes in [Ca]. Oligodendrocytes had an average resting [Ca] of 73 ± 20 nM. Contact with CNS myelin caused a relatively rapid [Ca] increase. The average maximum [Ca] level reached after contact with CNS myelin was 431 ± 273 µM. The [Ca] increase was reduced but not blocked by 5µM EGTa, which supports the idea that at least part of the [Ca] increase was due to a release of calcium from internal stores. A similar [Ca] increase was induced by contact with PNS myelin. These results suggest that oligodendrocytes might be able to recognize and react to specific molecules on the surface of other oligodendrocytes. This type of cell-cell interaction might play a role in preventing overlap of processes of adjacent oligodendrocytes at the developing Node of Ranvier. (Supported by the American Paralysis Assoc., the Marine Biological Laboratory at Woods Hole, and N.I.H.)


The role of phagocytic cells may be key in understanding the mechanisms involved in immune-mediated demyelination in the central nervous system. It has been hypothesized that myelin basic protein (MBP) may be the antigen most likely to be processed and presented by MHC class II molecules to elicit an immune mediated response. Although MBP has been used extensively in vitro and in vivo, the mechanism of phagocytosis and processing has not been characterized. We have used MBP-transfected Chinese hamster ovary (CHO) cells, a human histiocytic lymphoma cell line, U937, and human microglia to investigate the phagocytosis of MBP. To be able to follow MBP ingestion, we modified MBP with an antibody to the human cell line, anti-human MBP antibody, U937 and microglia were shown to ingest MBP both by flow cytometry and confocal microscopy. Activated EBV-B cells were shown to be the most efficient at phagocytosis of MBP as demonstrated by both 14C-MBP and FITC-MBP uptake. U937 and microglia were shown to ingest MBP albeit at slower rates. All three cell types failed to ingest MBP when incubated at 4°C, a characteristic of phagocytosis rather than pinocytosis. The rate and extent of MBP phagocytosis was markedly lower than that of cecile beads and bovine serum albumin. The processing of MBP is associated with the endosomal fraction of U937 after 2 hours and EBV-B cells after 8 hours. This is consistent with processing for binding to MHC class II molecules.

Two types of benzodiazepine (BZD) receptors have been characterized in brain: central (C) and peripheral (P). These receptors are encoded by different mRNA species and represented by distinct binding sites in brain membranes. BZD agonists and antagonists have different profiles of selectivity for these receptors. We have identified in brain membranes a BZD site with characteristics of a peripheral receptor. This binding site is present in brain membranes but not in membranes of cultured astrocytes from neonatal rat cerebral cortex. In homogenate cell preparations, the selective BZD ligands, [3H]PK 11195 and [3H]Ro5-4864, labeled high affinity sites (KD=0.82 and 2.4 nM, respectively) that appear to display a partial overlap. The maximal number of sites (Bmax) labeled with [3H]Ro5-4864 in brain membranes was not different from that labeled with [3H]PK 11195 (1.8 pmol/mg protein). Unlabeled Ro5-4864 competed for [3H]PK 11195 binding sites in a biphasic manner, suggesting the existence of PBZD sites that are not readily accessible to Ro5-4864. BZD ligands that bind to both C and P BZD receptors competed for [3H]PK 11195 and [3H]Ro5-4864 binding in a monophasic manner, and with similar affinities. These findings suggest that Ro5-4864, but not other BZD ligands tested, distinguished between putative subtypes of BZD receptors. Subcellular distribution studies indicated no apparent difference between the localization of [3H]Ro5-4864 and [3H]PK 11195 binding sites. The mitochondrial fraction of astrocytes revealed a lower or absent site in the binding of the two BZD ligands tested. This study suggests the existence of putative subtypes of BZD receptors in astrocytes, and indicates that their primary localization in these cells is in mitochondria.

BENZODIAZEPINE RECEPTOR IN CULTURED ASTROCYTES. T.J. Langan* and M.C. Slater, Dept. of Neurology, SUNY Sch. Med., Buffalo NY 14222.

Polypeptide growth factors (PGFs) generally act in sequential pairs: competence factors in early G1, and progression factors, particularly insulin (INS), in mid- to late G1. Newborn rat astrocytes were synchronized by the addition (at time 0) of 10% serum to cultures transferred to a defined medium (DM) containing peptides has been isolated and characterized from Lymnaea. The FMRFamide gene in Lymnaea contains two major peptide-encoding exons. One encodes the peptides FMRFamide and related peptides. The other encodes GDPFLRFamide and related peptides. Each exon encodes a peptide-encoding exon which is spliced onto the GDPFLRFamide encoding exon. One encodes the peptides FMRFamide and related peptides. One encodes the peptides FMRFamide and related peptides. The other encodes GDPFLRFamide and related peptides. The other encodes GDPFLRFamide and related peptides. In collaboration with David Price, five of the seven FLRFamide-containing peptides have been isolated and characterized from Lymnaea elegans (Rosoff et al., J. Neurosci., in press). Two different transcripts are produced as a result of alternative splicing; analysis of the corresponding cDNA sequences indicates that the two transcripts are developmentally regulated, reverse transcription/PCR experiments of staged RNA are currently being performed.


Norepinephrine (NE) inhibits the induction by IFN-g of class II antigen expression in cultured astrocytes (Frohman et al. PNAS 85:1292, 1991). We examined whether NE would also inhibit the LPS-induction of NOS in astrocytes as determined by nitrite accumulation. Primary astrocyte cultures exposed to LPS (500 ng/ml, 24 hr) but not NE (100 μM, 24 hr) increased NOS activity 15-fold over basal levels. However, NE elicited a dose-dependent inhibition of induction by LPS, with a threshold of 1 mM and maximal inhibition of 50-80% at 100 μM. Exposure to NE (100 μM) for 10 min was as effective as 24 hr continuous exposure in decreasing LPS-induction. In contrast, NE did not alter NOS activity pre-induced in astrocytes, suggesting that NE inhibition occurs at the levels of transcription not by protein modification. The induction of NOS by LPS was also blocked by the β-adrenergic agonist isoproterenol (10 μM) but not by the α-agonist phenylephrine. The response to NE was partially reversed by the β-antagonist propranolol, but not by the mixed α-agonist phenoxybenzamine. VIP (1 μM), but not NPY (1 μM), could also inhibit the induction due to LPS. These results suggest that induction of glial NOS by LPS can be regulated by catecholamines acting upon astrocyte β-receptors via elevation of cAMP levels. Conceivably the release of neurotransmitter in vivo may modulate the expression of inducible NOS in astrocyte possibly in response to injury or disease.


The FMRFamide gene in Lymnaea contains two major peptide-encoding exons. One encodes the peptides FMRFamide and related peptides. The other encodes GDPFLRFamide and related peptides. Expression of these two exons is mutually exclusive in individual cells, however both exons are spliced onto a common hydrophobic leader sequence. Cytoplasmic expression of these two peptide-encoding exons is regulated by differential RNA splicing. We have recently found that the FMRFamide gene contains at least two other peptide-encoding exons which are spliced onto the GDPFLRFamide exon in the messenger RNA. One of these exons contains the tetrasaccharide cleavage sequence RERK and also encodes a novel peptide SKF/MRFamide. Genomic sequence of 5783 base pairs has been determined and, on comparison with cDNA sequences, shows that the FMRFamide and GDPFLRFamide exons are separated by an intron of 2914bp. The mRNAs encoding FMRFamide and GDPFLRFamide are 1647bp and 1623bp respectively. By the use of immunocytochemistry, protein purification and sequencing we have shown that each exon is translated and processed. This work is supported by the SERC.
545.3

The generation of a master set of the cDNAs expressed in the human brain, knowledge of the chromosomal location of each cDNA and the complete DNA sequence of the protein coding region of each expressed brain gene would constitute a tremendous resource to human biology and neuroscience. Our goal is to significantly contribute to the development of such a resource through the large scale collection, analysis, and mapping of human brain. We have developed a method for enriching for cDNAs that are unique from one another, and have refined strategies for automated single-pass and full-length sequencing of each cDNA and for protein mapping of each cDNA to a location in the genome. We have also identified a subset of cDNAs that contain polymorphic microsattelite sequences and demonstrate how they can be converted to highly informative (PIC value > 0.7) gene-associated genetic markers. Single-pass sequencing data from the cDNAs is being used to search DNA and protein databases using the BLAST programs. Present, most of the sequenced cDNAs correspond to potentially new human brain genes, while a significant number of cDNAs appear to represent human homologs of interesting genes found in other species. Among these is the Descemtes brain gene, a regulator of a number of homeotic genes, several cDNAs related to known neurotransmitter receptor genes, ERK3, an extracellular signal regulated kinase and a potentially novel sodium channel gene. Currently in our laboratory automated single-pass sequencing is being carried out at a rate of several thousand cDNAs per year. Therefore, coordination of this effort with other laboratories doing similar work should permit the sequence identification of most of the genes expressed in the human brain within the next few years.

545.5

Protein kinases are key intermediates in stimulus-induced neuronal responses. However, the synaptic stimuli needed to activate specific kinases is unclear. Using primary cultures of cortical neurons, we have investigated the synaptic activation of Ca2+-calmodulin-dependent protein kinase (CaMKII) and mitogen activated protein kinase (MAPK). Burst of spontaneous synaptic activity were induced and monitored with Ca2+ indicators. Phosphorylation of these kinases during their activation allows activity to be preserved and assayed in cell extracts. We report that although both these kinases are activated by synaptic activity, they have different kinetics. While 90% of maximal CaMKII activation was observed after only 10 s of burst activity, MAPK is not affected at this early time and is only activated to 30% of maximal after 2 min of stimulation. In parallel experiments, we examined the rate of decay of activity following synaptic stimulation. The half life of stimulated CaMKII was 10-30 s, while MAPK decayed by 50% within 6-10 min. These data provide evidence for differential regulation of these kinases by synaptic activity.

We thank H. Schulman and N. MacDonald for advice and reagents.

545.7
CH2 ZINC FINGER GENES EXPRESSED IN THE MOUSE NERVOUS SYSTEM. N. Mazarakis, N. Galant, J. Brockes,* and F. Grosveld. Lab of Gene Structure and Expression, National Institute for Medical Research, The Ridgway, London NW7 1AA, UK; *Institut National de la Sante et de la Recherche Medicale, VPF SHEF, UK.

The CH2 zinc finger motif is a structure that contains highly conserved pairs of cysteine and histidine residues first identified in the Xenopus transcription factor TFIIH. It constitutes a sequence spanning an invariant domain that is found in a superfamily of invertebrate and vertebrate genes. Some of these genes encode mRNAs of activator factors with regulatory roles in cellular growth and differentiation. In an attempt to study the regulation of neuronal differentiation a mouse brain cDNA library was screened under low stringency conditions with a zinc finger probe from a mouse gene that was shown by Nothern blot analysis to generate a 3.7 kb brain specific transcript present during neurogenesis. In situ hybridization studies indicated that this message had a paraneuronal pattern of expression. A large pool of cDNA clones was isolated that contained this and other highly homologous CH2 zinc fingers. At least two novel CH2 zinc finger genes have thus been identified. These data indicate that several members of the zinc finger family are being expressed in the developing mouse nervous system.

545.4
TETRIC POTENTIATION ALTERS THE ABUNDANCE OF SEVERAL mRNAs IN SINGLE LIVE CA1 HIPPOCAMPAL NEURONS. J.A. Mackler*, B.P. Brooks and J.H. Eisenberg. Univ of Phila PA 19104.

We examined the effects of use-dependent changes in synaptic transmission on the relative abundances of mRNAs of several proteins in single individual neurons. The approach combined whole-cell recordings in rat hippocampal slices with amplification of the polyA+ RNA population from single CA1 cortical neurons. We examined the relative abundance of all of the reagents necessary for cDNA synthesis and the polyA+ RNA was amplified using the T7 RNA polymerase promoter sequence and SP6-CTP.

Screening of multiple candidate cDNA neuronal clones with the amplified probe revealed several differences in relative mRNA abundances between control and TTX treated animals or more after potentiation of evoked e.p.p.s. A 300% increase in CamKII mRNA occurred along with a 50% decrease in protein kinase C (PKC) mRNA. This 5-fold change in mRNA levels may accentuate the activity of the CamKII second messenger signal in comparison to PKC. Phospholipase A2 mRNA was unaffected. A 2-fold increase for zif-268 mRNA, without consistent changes in c-fos or c-jun and HSP70, suggests that transcriptional activators are selectively activated by synaptic use. The above changes were prevented by the prior addition of APV, a NMDA receptor antagonist.

The total of the described changes of mRNA levels from individual cells provide a detailed picture of a neuron after synaptic potentiation and in the context of synaptic connectivity and glut association.

545.6

One of the most fascinating issues in the study of the developing and adult CNS is transcriptional regulation of genes. POUs proteins are trans-acting factors which contain two highly conserved DNA binding domains (POU domain and NIH-POU-specific domain). We used in situ hybridization histochemistry to examine the distribution of mRNA encoding four Class III POU genes recently cloned in our lab (PNAS 89:3280; PNAS 89:3285). Serial adjacent sections from adult female Swiss-Webster mice were studied. For Brain-1, Brain-2, and SCIP, we used [3S]-labelled riboprobes (GRNA) targeted to the 5' end of the coding sequences and the adjacent untranslated region of each transcript. For Brain-4, the probe was targeted to the mRNA encoding the translated region 5' to the POU-specific domain. The sense strand of the Brain-4 probe was used as a negative control.

Brain-1 was the most abundant transcript of the four transcripts, being especially prominent in the piriform cortex, olfactory tubercle, and lateral ventricular ependyma. Brain-2 was prominent in the piriform cortex, vertical limb of the diagonal band of Broca, lateral ventricular ependyma, paraventricular and supraoptic hypothalamic nuclei, and substantia nigra, pars compacta. SCIP was abundant in the piriform cortex, striatum, accumbens nucleus, islands of Calleja, vertical and horizontal limbs of the diagonal band of Broca, nucleus of the lateral olfactory tract, CA1 and CA2 pyramidal cells of the hippocampus, medial habenula, and the inferior colliculus. Brain-4 was expressed in the olfactory bulb, striatum, nucleus accumbens, olfactory tubercle, lateral ventricular ependyma, medial habenula, and the paraventricular and supraoptic nuclei. All four Class III genes were expressed in laminar patterns in the neocortex. More detailed analysis is being pursued in the adult and developing mouse.

545.8

The mitochondrial enzyme cytochrome oxidase (CO) is a model for studying coordinate regulation of nuclear and mitochondrial gene expression. The enzyme contains 3 mitochondrial-encoded, and 10 nuclear-encoded subunits. We used in situ hybridization to study mitochondrial DNA (mtDNA), COI mRNA (mitochondrial-encoded), and CO4 and CO8 mRNAs (both nuclear-encoded), in the visual system of normal and 3-7 day monocular TTX-treateed macaques. In all animals, mtDNA and COI mRNAs were detected in neocortex while CO4 mRNA was not detected by in situ hybridization. However, CO4 and CO8 mRNA were mainly localized in perikarya, confirming our previous findings (J. Neurosci. 11:1942). Compared with normals, TTX-treated animals had not only decreased CO activity (shown histochemically) and CO protein (shown immunohistochemically), but also decreased mtDNA and subunit mRNA levels, in functionally deprived laminae of the lateral geniculate (LGN) and occipital dominance columns of area 17. After 7 days of TTX, mtDNA fell by 26%; CO1 mRNA by 49%; CO4 mRNA by 18%; and CO8 mRNA by 29%, as determined by counterimmunostained grain counting in LGN neurons. CO activity, measured densitometrically, decreased 23%. These results indicate that CO subunit mRNAs are disproportionately regulated in brain, subsequent to functional deprivation. The major acute control over CO activity is probably exerted by regulation of mRNAs for mitochondrial-encoded subunits, which form the catalytic core of the enzyme. (Supported by NIH grants NS18112 and EY05439 to MWR, and by an MCW MSTP fellowship to RFR.)
459.9
GENES WITH TRINUCLEOTIDE REPEATS: POTENTIAL CANDIDATES FOR NEUROPSYCHIATRIC DISORDERS. Robert C.A. Lott, S.S. McInnes, M.G. and Antonarakis, S.E. Departments of Psychiatry and Neuroscience, and Center for Medical Genetics, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Expansions of trinucleotide repeats (CGG or CTG) in mRNA underlie three neuropsychiatric disorders: Fragile-X syndrome, X-linked spinal and bulbar muscular atrophy, and Myotonic dystrophy. In addition, in unafflicted individuals, the number of repeats is highly polymorphic. In an attempt to find additional genes with trinucleotide repeats, we have screened a human cerebral cortex cDNA library with CGG and CTG repeat oligonucleotides. There were dozens of positives per large plate (~50,000 plaques per plate). Out of an initial ten clones, one was of myotonic dystrophy (Ea. et al. Science 255, 1256) with 13 CTG repeats, two were 28S ribosomal RNA and two were a human homolog of dog SRP (both of which are known to have repeats). In addition, two appear to represent repeat-containing cDNAs with novel sequences. In one (A3) and possibly both, the repeat is within an open reading frame. Genotyping using PCR across the repeat identified one fragment length polymorphism out of six individuals screened. We are now studying an additional series of clones, generated by screening the library at higher stringency, several of which appear to be novel sequences. Genes with trinucleotide repeats may provide markers with high polymorphic index for linkage studies and are potential candidate genes for neuropsychiatric disorders.

459.11
NAP, A HUMAN CEREBELLAR DEGENERATION ANTIGEN, IS A NOVEL, NEURON SPECIFIC, ADAPTIN FAMILY MEMBER. M.O. Mckeever AND R.B. Darnell. * Lab of Neuro-Oncology, Memorial Sloan Kettering Cancer Center, NY, NY 10021.

We have previously shown that antiserum from a patient with atypical cerebellar degeneration recognizes antigens expressed in cerebellar Purkinje cells and some neocortical neurons (Darnell et al., J. Neurosci. 1991;11:1224-1230). Using this antiserum we have isolated a clone from a human cerebellar expression library which, together with overlapping cDNA's, predict a protein sequence of 851 amino acids, of Mr. 96kDa. Antibody affinity purified with the cloned fusion protein recognized the same Purkinje antigens as native patient's antiserum. Sequence analysis revealed homology (31% identity over 296 amino acids) with the clathrin binding domain of the adaptin family of proteins. Northern blot analysis using human polycystic RNA demonstrated that this gene is expressed only in brain. These results identify a novel neuron specific member of the adaptin family, termed NAP, which is recognized by serum from a patient with human cerebellar degeneration. NAP may be involved in regulating neuronal specific aspects of vesicular trafficking, particularly receptor mediated endocytosis.

460.1
EXPRESSION OF POTASSIUM CHANNEL TRANSCRIPTS IN THE EMBRYONIC AMPHIBIAN NERVOUS SYSTEM. A.B. Ribera*. Department of Physiology C-240, University of Colorado Health Sciences Center, Denver, CO 80262.

Differentiation of the voltage dependent potassium current in amphibian spinal neurons is delayed with respect to the maturation of calcium current, regulating action potential duration. The molecular basis for this program of electrical excitability is being analysed using probes for voltage dependent ion channel genes. Maturation of potassium current is pivotal for action potential differentiation, and thus efforts have focused on this ion channel. A second Xnumpus potassium channel gene expressed in the developing nervous system has been cloned. This Xnumpus gene sequence is related to the mammalian sequences RCK1 and MBK1 and is thus called Xsh1a. RNAse protection assays indicate that Xsh1a is expressed in the embryonic nervous system at levels comparable to that of the previously reported potassium channel gene, Xsh2a. The predicted peptide has 490 amino acids. At the amino acid level, Xsh1a is 86% and 75% identical to MBK1 and Xsh2a, respectively. In the 3' untranslated region of Xsh1a, there is a region of 60 nucleotides that shows 70% similarity to sequences found in homologous locations in the 3' untranslated regions of MBK1 and RCK1. Functional expression of Xsh1a in oocytes induces a delayed rectifier potassium current. The induced current shows high sensitivity to the potassium channel blocker TEA, being reduced by >90% by 15 mM TEA.

The functional properties of Xsh1a in addition to its tissue and temporal patterns of expression of Xsh1a are consistent with its putative role in regulating excitability of developing spinal neurons.

Supported by NIH NS3217 and NS21531.

460.2
ALTERED POTASSIUM CHANNEL GENE EXPRESSION AND NEURAL DIFFERENTIATION. S. M. Jones* AND A. B. Ribera. Department of Physiology C-240, University of Colorado Health Sciences Center, Denver, CO 80262.

Several voltage-dependent potassium currents are expressed in embryonic vertebrate neurons and their differentiation regulates the development of action potentials in amphibian spinal neurons. Xsh1 and Xsh2 are two Xenopus Skg-like potassium channel genes that are normally expressed in the embryonic nervous system. In order to determine how the program for electrical excitability is established, Xsh1a and Xsh2a were overexpressed in developing embryos.

Overexpression was achieved by injecting capped Xsh1a or Xsh2a transcripts prepared in vitro into 1 cell of a 2 cell stage embryo. Rhodamine-conjugated dextran was included in the injection solution to follow the progeny derived from the original injected cell. Inclusion of potassium channel RNA, but not β-galactosidase RNA, in the injection solution led to a dramatic decrease in the number of morphologically differentiated neurons in vitro that are rhodamine fluorescent. This effect was dose dependent; at concentrations less than 0.2 µg/ml, the potassium channel RNA lost its effectiveness. Neurite extension in whole mount embryos was examined immunocytochemically with acetylated α-tubulin antibodies; these studies indicated that at concentrations greater than 0.2 µg/ml, overexpression of potassium channel RNA led to abnormal motor neuron development.

Electrophysiological analysis of potassium currents in fluorescent and nonfluorescent neurons prepared from embryos injected with this low dose of potassium channel RNA did not reveal any difference in current densities. We are now studying the effects on current density of injection of higher concentrations of Xsh1a or Xsh2a RNA. Supported by NIH NS3217 and NS21531.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
460.3 REGULATION OF mRNA FOR THE Kv3.1 POTASSIUM CHANNEL IN VITRO AND IN VIVO. T.M. Deretic*, X. Li, S. Karpe, L.K. Kazmerski and N.C. Birnberg, Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Transfection of AIT20 cells with the H-Havon oncogene causes a decrease in the width of action potentials and is associated with the induction of the potassium channel, KvK3.1 (Hennemick et al., J. Neurosci., 1992). In this study we have examined the effects of transmembrane signalling pathways on Kv3.1 gene expression in wild-type (WT) and rat transformed (R1) AIT20 cells. Long-term treatment (24-48 hrs) of WT cells with 10 ng/ml basic fibroblast growth factor (bFGF) resulted in the induction of Kv3.1 mRNA. bFGF treatment also caused a substantial decrease in action potential width consistent with a role for Kv3.1 mediated currents. In contrast, short-term treatment with 5 mM dibutyryl cAMP or 50 mM KCl increased Kv3.1 mRNA levels in both WT and R1 cells. The effect of depolarization was inhibited by the Ca2+ channel blocker, nimodipine, suggesting that the influx of Ca2+ is important in regulating Kv3.1 expression. Interestingly, R1 cells treated with high K+ or dibutyryl cAMP displayed a transient decrease in Kv3.1 mRNA levels at 2-8 hr. Both of these treatments caused a rapid induction of the e-Fos gene and an increase in AP-1 DNA binding activity. It is possible, therefore, that the Kv3.1 gene is regulated in a biphasic manner, strongly through the actions of second messengers and delayed through the actions of immediate early genes. Because these same signaling pathways may also be activated by neuronal activity, we examined the effects of seizures on Kv3.1 gene expression. We found that 6 hr after a tetrazole-induced seizure levels of Kv3.1 mRNA were decreased approximately 50% in rat hippocampus. We are currently investigating the long-term effects of seizure activity on the expression of the Kv3.1 gene.

460.4 STABLE EXPRESSION OF A RAT BRAIN K+ CHANNEL (K.3.1) IN HUMAN EMBRYONIC KIDNEY CELLS. S.D. Critch, B.A. White, D.J. Amott, H.S. Lopez, and A.M. Brown, Dept. of Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX 77030.

Biophysical analysis of K+ channels is often made difficult by the diversity of K+ channels found in cells. To address this issue, we have begun to express individual K+ channels using stable transfection of human embryonic kidney (HEK 293) cells. In this study, the biophysical properties of a stable K+ channel, K3.1, were compared to those produced by transient expression following the introduction of K3.1 cDNA into Xenopus oocytes. The region of DNA coding for the K+ channel K3.1 was ligated to a mammalian vector, pRCMV (Invitrogen B), which contained a polylinker region, a cytomegalovirus promoter and a Neomycin resistance and K3.1 DNA was inserted into the pRCMV vector. The K+ channel was incorporated by lipofection (Lipofectin®; BRL) at 1 μg DNA/17 KHEK 293 cells. Twelve out of 15 antibiotic-resistant clones randomly selected for biophysical analysis contained detectable levels of voltage activated inward current (10.9 ± 2.2 nA at +90 mV, n=6) in these cells. The reversal potential of HEK 293 K3.1 shifted in Neristan manner with external [K+]i concentration. Moreover, the K+ currents were sensitive to concentrations of TEA (IC50 165 μM) that block K3.1 channels in Xenopus oocytes. The voltage-dependence of activation of K+ in HEK cells was similar to that observed in the oocytes (V1/2 ≈ 55 mV). The voltage-dependence of inactivation appeared to be different, however (+11 ± 2 mV vs +20 mV, respectively). Single channel data indicate a unitary conductance of approximately 25 pS, comparable to the 21 pS channel that is expressed in Xenopus oocytes. HEK 293 cells are suitable mammalian system for the expression of K+ channels and may provide insights into the posttranslational modification of these channels. (Support: F33-MS08579 SDC, NS23877 AMD).

460.5 DIFFERENTIAL EXPRESSION OF K+ CHANNEL mRNAs IN THE RAT BRAIN AND DOWN-REGULATION IN THE HIPPOCAMPUS FOLLOWING SEIZURES. M.T. Tsiour, M. Sheng, D.B. Lowenstein, T.N. Jan & L.Y. Jan*. Howard Hughes Medical Institute and Departments of Physiology and Biochemistry, Univ. of California, San Francisco, San Francisco, CA 94143.

K+ channels are major determinants of membrane excitability. Differences in neuronal excitability within the nervous system may arise from differential expression of K+ channel genes, regulated spatially in a cell-type specific manner, or temporally in response to neuronal activity. We have compared the distribution of mRNA for three K+ channel genes, Kv1, Kv2 and Kv4 in rat brain, and looked for activity-dependent changes following treatment with the convulsant drug pentyleneetetrazole. Both regional and cell-type specific differences in K+ channel expression were found. In addition, neuronal activity caused a reduction of Kv1 and Kv2 mRNA in the dentate granule cells of the hippocampus, raising the possibility that K+ channel gene regulation may play a role in long-term neuronal plasticity.


Transcripts of the rat KsH11.1 gene, one of the Shaker III gene family, express delayed rectifier voltage-dependent currents in Xenopus oocytes. We and others have previously reported the isolation of cDNAs encoding several alternatively-spliced versions of this transcript and of other genes of this subfamily predicting proteins differing in their carboxyl ends. The Drosophila Shaker gene encodes alternatively-spliced transcripts with different 3' and 5' coding regions. We now report the isolation of two types of transcripts of the KsH11.1 gene with divergent untranslated regions (5' UTR). The 5' sequences differ up to a point 5 bases upstream of the putative start codon (T5'). The presence of an AG the dinucleotide characteristic of the exon boundary of 5' splice junctions, at position 4, is suggestive of the presence of these different 5'UTRs result from alternative splicing. Unlike the fly Shaker gene, these alternative exons encode only untranslatable sequences. Northern blot analysis of rat brain mRNA with probes specific to KsH11.1 show a complex banding pattern that is not resolved by the utilization of probes specific for the 3' alternatively-expressed exon of this gene. However, Northern blots hybridized with probes specific to the two alternatively-spliced 5' UTR exons, designated alpha and beta, produce distinct banding patterns. On the basis of both Northern blot analysis and corroborating in situ hybridization studies, alpha is more highly expressed than beta in the rat CNS. As it is thought that 2 UTRs are involved in regulation of the processing and translation of mRNA, these results raise the intriguing possibility of differential post-transcriptional regulation of K+ channel expression at the RNA level.

460.7 COMBINATORIAL ASSEMBLY OF THE EAG POLYPEPTIDE WITH K+ CHANNEL SUBUNITS FOR MEDIATING cGMP-DEPENDENT MODULATION OF K+ CURRENTS. X. Zhong and C.C. Wu. Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242.

Co-expression of different (Shaker) 2B subunits in the Xenopus oocyte has confirmed the oligomer assembly of the voltage activated 2B channels. However, the transient current I4, in Drosophila muscles is affected not only by SA mutations but also by those of the outer 2-go (ego) locus, which encodes a polypeptide homologous to but distinct from SA subunits. This suggests participation of products from different genes in the assembly of native 2B channels. Existence of multiple SA and 2B alleles enabled the testing of this idea in various double-mutant combinations and allowed an investigation of the functional role of different types of subunits in the channel. Voltage-clamp analysis of amplitude, time course, and steady-state inactivation revealed that the effect of eg2, egm1, egm2, eg24 on I4 varied with 2B alleles in the background. For instance, the amplitude of I4 is increased in eg2 2B, decreased in egm2 2B, but unchanged in eg24 2B as compared to each of the corresponding 2B background. The allele-specific interactions in eg 2B double mutants reflect a close association between the 2B and eg subunits within the I4 channel. Since eg2 subunits affect the peak, activation, and inactivation kinetics, including Drosophila-activated delayed Ig, the eg subunit may be present in multiple 2B channels. The functional significance of such heteromultimeric assembly of K+ channels is suggested by our in vitro inositol 1,4,5-trisphosphate (IP3) and cGMP (2 mM) and ino analogs, 8-BrcGMP (500 μM) and dibutyryl cGMP (500 μM), is either altered or eliminated by different eg mutations. The role of the eg subunit is channel modulation, as opposed to that of 2B subunits in determining the channel gating and conductance, supports the notion that at least two classes of subunits, each dedicated to different functions, co-assemble into a variety of K+ channel complexes.

460.8 MEMBERS OF A MOUSE SUBFAMILY OF GENES ENCODING VOLTAGE-GATED POTASSIUM CHANNEL SUBUNITS FORM HETEROMULTIMERS WHEN COEXPRESSION IN XENOPUS OOCYTES. Darin Herschlag and B.L. Tempel. Dept. of Pharmacology and Medicine, Univ. of Washington & VA Medical Center, Seattle, WA 98109.

Potassium channels with divergent functional properties could arise, in principle, by the aggregation of nonidentical subunits. We wished to determine if heteromultimer formation was possible for the products of MK1 and MK4 mouse genes, members of the mammalian Shaker-like subunits, and the two human potassium voltage-gated potassium channels. We also sought to test the hypothesis that potassium channels are comprised of 4 subunits. When MK1 and MK3 were expressed in Xenopus oocytes using the two-electrode voltage-clamp technique, we observed potassium currents that differed in their sensitivity to fraction I of dextrotoxin (DTX-I). The K's for DTX-I in oocytes expressing MK1 (N=7) and MK3 (N=8) were 3 and 45233 mV, respectively. We exploited the large difference in DTX-I sensitivity to test if heteromultimers comprised of MK1 and MK3 subunits form when coexpressed in oocytes. When MK1 and MK3 were coexpressed in several different ratios, the DTX-I sensitivities of the resulting currents were always greater than would be predicted by an additive model based on the hypothesis that MK1 and MK3 form only homomultimers. With a MK1:MK3 = 1:3 ratio, the K was 14 mV (N=4), which is much less than the K of 3100 mV predicted by the additive model. This suggests that heteromultimers composed of MK1 and MK3 subunits with intermediate DTX-I sensitivities can form in oocytes and that they have DTX-I sensitivities much closer to MK1 than to MK3. This implies that one MK1 subunit confers nearly full DTX-I sensitivity on a channel complex. The approach of MacKinnon (Nature 355: 252, 1991) was used and the results were consistent with a channel complex composed of 4 supported by NIH NS27206 and the VA.
**460.9**

**FUNCTIONAL INTERACTIONS BETWEEN SUBUNITS OF A CHIMERIC K+ CHANNEL.**

G.E. Kincaid, J.A. Drewe, M. DeBlaey, M. Tagliatela, H.A. Artmann, and A.M. Brown. Departments of Anesthesiology and Molecular Physiology and Biophysics. Baylor College of Medicine, Houston, TX 77030.

We have shown previously that in a chimeric K+ channel (22 pS K+ conductance, expressed in Xenopus oocytes) the non mutation V369l decreases channel open time and causes fast inactivation, without affecting conductance (De Biase, this meeting). L374V shortens channel open time and markedly reduces conductance (5 pS) with little effect on inactivation. Furthermore, the double mutation V369l+L374V increases channel open time and produces an intermediate conductance (10 pS). Here we show that the fast inactivation produced by V369l is abolished in the double mutant by increased burst duration. We tested for functional interaction of non-consecutive pore residues across subunit boundaries by co-injecting a mixture of rRNAs encoding the point mutants V369l and L374V. Two major classes of channels with intermediate conductances (8 and 14 pS) were observed in addition to the expected homotetrameric channels. Unlike the short-opening homotetramers, both classes of putative heterotetrameric channels had burst durations and open times approaching those of the double mutant. These results suggest that pores residing in adjacent subunits form a closely packed structure which determines both ion conductance and stability of the open state of the channel. Supported by NIH grants NS29473 (GEK), and NS23877 and HL37044 (AMB).

**460.10**


Spatial segregation of voltage-sensitive ion channels in surface membranes of neurons and muscle fibers is critical to the conduction of electrical impulses. We have begun to investigate the molecular mechanisms that govern the restricted expression using the drk1 (K.C.1) K+ channel polypeptide as a model. We have expressed wild type and mutant drk1 polypeptides in polarized and nonpolarized cells by transfecting drk1 cDNAs into fibroblast (COS), epithelial (MDCK) and neuronal (PC12) cell lines, and have begun to characterize the targeting and functional characteristics of these drk1 polypeptides in these different cellular backgrounds. Expression of wild type and mutant drk1 polypeptides in COS cells is highly efficient, with the majority of expressed polypeptide found on the cell surface as judged by: 1) vectorial labelling with membrane impermeant biotinylated reagents; 2) plasma membrane localization using immunostaining; 3) presence of large (20 nA = 500 pA/P) whole cell K+ currents. Studies with membrane impermeant, reducible crosslinking reagents show that both wild type and mutant drk1 polypeptides form multisinus channel complexes on the cell surface. MDCK and PC12 cell lines stably expressing drk1 polypeptides have been generated. Studies to determine the cell surface localization of drk1 in these cells are in progress.

**460.11**

**SUBCELLULAR SEGREGATION OF A-TYPE POTASSIUM CHANNELS IN RAT CENTRAL NERVOUS MORGAN SHENG, MOE-LING TSAUR, YIH YUN JEN, and LILY Y. JEN Howard Hughes Medical Institute. Dept. of Physiology and Biochemistry, Univ. California, San Francisco, CA 94143-0724.

In the mammalian nervous system, K channels regulate diverse aspects of neuronal function and are encoded by a large set of genes. The roles of the many different K channels that are expressed in individual neurons could be dictated by their localization to specific subcellular domains. Using gene-specific antibodies, we show that two K channel polypeptides, Kv1.4 and Kv4.2, that are found in the A-type currents when expressed in Xenopus oocytes, are segregated in rat central neurons. Kv1.4 is targeted to axons and possibly terminals, while Kv4.2 is concentrated in dendrites and somata. Translation of both proteins, however, occurs in the cell body. The differential distribution implies distinct roles for these channel proteins in vivo; their localizations suggest that Kv1.4 and Kv4.2 may regulate synaptic transmission via presynaptic, or postsynaptic mechanisms, respectively.

**460.12**

**CONTRASTING IMMUNOHISTOCHEMICAL LOCALIZATIONS IN THE RAT BRAIN OF TWO NOVEL K+ CHANNEL SUBFAMILIES.** P.M. Hwang, M. Pouli, D.S. Britt and S.H. Dreid, Department of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

A number of mammalian voltage-dependent K+ channels with closely similar amino acid sequences and electrophysiological properties have been cloned; however, little is known about how multiple K+ channels are arranged to serve similar functions. We have recently identified, cloned and characterized a novel K+ channel, designated CDRK1 (Hwang et al., Neuron 9:473-481, 1992). CDRK1 appears to be a member of the Drosophila Shab K+ channel subfamily, most closely resembling DRK1 (Froeh et al., Nature 349:624-625, 1989), the only other rat homolog of Shab. Electrophysiological analysis of expressed CDRK1 reveals delayed rectifier properties similar to those of DRK1. We now have made synthetic peptide antibodies specific for CDRK1 and DRK1 proteins. Remarkable contrasting immunohistochemical localizations in the brain as well as in peripheral tissues have been observed for both channels, suggesting that these highly homologous K+ channels with distinct localizations may play uniquely important roles in the control of membrane potential in various excitable tissues.

**PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING**

**461.1**


The goal of this study is to study the possible central and peripheral mechanisms for the sprouting of primary afferent fibers in the partially denervated spinal cord.

The first group of adult Sprague-Dawley rats (n = 5) received unilateral dorsal root ganglionectomies of L4-L6 on one side and rhizotomies of L1-L4 dorsal roots on the other side. The second group of rats (n = 3) received unilateral dorsal root rhizotomies of L1-L4 dorsal roots on the right side, followed seven weeks later by contralateral rhizotomies. Two months after the first surgery, all rats were anesthetized, and stimulated by immersing both of their hindlimbs in 32 °C water for 20 seconds. The rats were perfused two hours later. CGRP and c-fos immunocytochemical stains were performed.

Preliminary results indicate that CGRP immunoreactive fibers had sprouted into a new area (laminar III) on the chronic ganglionectomized sides. Also, the area of CGRP immunoreactive fibers and the area occupied by c-fos immunoreactive cells were much greater on the ganglionectomy/dorsal rhizotomy sides in the first group of rats, and much greater on the chronic side in the second group of rats. These results suggest both central and peripheral denervation contribute to the sprouting of thinly and unmyelinated primary afferent fibers in the partially denervated spinal cord. Furthermore, combined central and peripheral denervation (via ganglionectomy/dorsal rhizotomy) induces sprouting of thinly and unmyelinated fibers into novel regions. Supported in part by NS18913 and NS27511.

**461.2**


The retinal ganglion cells (RGCs) of embryos and axons, as well as those of the marginal retinal growth zone in adults, carry E21 staining over their entire surface. On mature RGCs, E21 staining is restricted to contact sites between the cells and to the zone of RGC axon terminal arbors in the tectum. After optic nerve transection the regenerating axons re-express Neurolin throughout their path while the RGCs keep it restricted to their contact sites. The NH2-terminal aminoacid sequence of Neurolin has similarities to that of the recently discovered cell adhesion molecule DM Grasp (Burns et al., Neuron 7, 1991) and SCI (Tanaka et al., Neuron 7, 1991) of the chick spinal cord.

In fact, E21 also stained motoneurons, floorplate cells and the DRG entry zone and tract in the developing goldfish spinal cord and only the latter in adults.

Neurolin may represent either the goldfish homologue of DM-Grasp/SCI or another member of that family.
461.1

ASTROCYTES DEMONSTRATE REGIONAL DIFFERENCES IN PROMOTING DENDRITIC GROWTH. P. Lee Roos* and T. Reh. Depts. of Neurosurg. and *Biol. Struct., University of Washington, Seattle, WA 98195.

Glia from different regions of the CNS have been shown to be heterogeneous in their ability to promote neurite outgrowth from neurons. Hence it is known whether glia from different CNS regions differ in their abilities to promote axons vs dendrites from neurons. Therefore we compared total dendritic and axonal growth from embryonic mouse cortical neurons (E 18) grown in vitro on postnatal day 1 (P4) astrocytes purified from cortex, mesencephalon, striatum, olfactory bulb, spinal cord (GFAP>90%); Muller glia from retina; and fibroblasts. Double immunolabelling (MAP2, M6, NF-H) was used to identify mouse cortical dendrites and axons after 5 DIV. While axon length was similar on all glial monolayers, dendritic growth was nearly threefold greater on cortex, retina and olfactory bulb than from other areas of the CNS. These findings demonstrate regional differences in the ability of astrocytes to support dendrogenesis. Supported by NIH NS 30305.

461.5

ACTIVITY-SENSITIVE SIGNALLING BY INSULIN-LIKE GROWTH FACTORS IN THE DEVELOPING AND REGENERATING NEUROMUSCULAR SYSTEM. P. Garner* and C. Schneider, Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel.

Synapse elimination during development and nerve sprouting in the adult affect the arrangement of presynaptic terminal branches and are coupled to post synaptic activity. Thus, the prolonged absence of postsynaptic activity leads to sprouting in the adult and prevents the retraction of collateral branches. In the neuromuscular system, lack of muscle activity leads to a rapid elevation of muscle fiber-derived IGF1 in the adult and prevents the developmental downregulation of muscle IGFs during synapse elimination. We have searched for motoneuron mRNA's and proteins whose levels are controlled by muscle activity and have determined whether muscle-derived IGFs are sufficient and/or necessary to mediate activity-sensitive retrograde signalling from muscle to spinal motoneurons. We report that: 1) the mRNA coding for the growth-associated proteins (GAPs) GAP-43 and tubulin-α are downregulated at rat and chick spinal motoneurons at the onset of the synapse elimination process; levels of terminal-associated GAP-43 decline during the elimination process; 2) motoneuron GAP downregulation is controlled by the periphery; it is prevented by local muscle paralysis or by elevated levels of IGFs in the muscle; 3) muscle-derived IGFs accumulate at the neuromuscular junction and are taken up and transported in motor nerves; 4) blockade of IGFs in the muscle by local applications of recombinant IGF binding protein prevents the effect of paralysis on motoneuron GAP downregulation and on the pattern of intramuscular nerve growth. Therefore, IGFs mediate activity-sensitive retrograde signalling from suletal muscle to spinal motoneurons.

461.7

EFFECT OF CTNF ON NEURITE OUTGROWTH FROM CULTURED SPINAL NEURONS. N.M. Oyenika* and D.L. Wigston. Program in Neuroscience and Dept. of Physiology, University School of Medicine, University of North Carolina at Chapel Hill, NC 27599.

There is a serious need for agents that may promote axonal growth after spinal cord injury. Although ciliary neurotrophic factor (CNTF) can enhance the survival of a class of spinal neurons, the influence on neurite outgrowth or regeneration of axons from motoneurons or other spinal neurons is not known. To investigate the effect of CNTF on neurite outgrowth from spinal neurons, we dissociated motoneuron cultures from the lumbar spinal cord of rats at stage 30 (E16) chick embryos and plated them on laminin-coated glass coverslips in medium with or without CNTF (recombinant human CNTF; Sigma). By 48 hr, we found that neurites were about 65-100% longer and the total neurite length per neuron was 60-150% greater in cultures exposed to CNTF (p<0.025). In addition, CNTF enhanced neuronal survival. The enhanced neurite outgrowth was not a consequence of the higher cell density in CNTF-treated cultures; unlabeled cells that were plated so that their final density matched that of CNTF-treated cultures did not show enhanced neurite outgrowth. To examine the effect of CNTF on subpopulations of spinal neurons, we found that cultured cultures derived from the ventral spinal cord which contains motoneurons, with cultures derived from the dorsal spinal cord which lacks motoneurons. CNTF enhanced neurite outgrowth to about the same extent in ventral and dorsal cultures. We also identified motoneurons in some experiments by labelling them retrogradely with diI. The effect of CTNF on neurite outgrowth was similar for labelled and unlabelled neurons, and was therefore not specific to motoneurons.

We conclude that CNTF promotes neurite outgrowth from spinal cord neurons in vitro. CNTF may have a similar effect on neurons in the injured spinal cord. (Supported by the American Paralysis Association.)

461.8

SKEROTONIN AND ACETYLCHOLINE ALTER BRANCHING DECISIONS AND Soma SIZE OF DEVELOPING LIECH REZUS NEURONS. S.M. Elgar and U.S. Stan* Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

Embryonic axons of Reticular neurons (Rs), one of 5 types of SHT neurons in leech spinal ganglia, show a stereotyped sequence of pathway and branching decisions. Since synthesis of 5-HT receptors for 5-HT and ACH appear early in Rz axon outgrowth, we asked whether 5-HT or ACH helps to shape these decisions. In Thoropyrgus, whose normal sequence of Rs axonal branching differs from that previously described in Hirudo, the primary axon forms a T-branched; the posterior arm of the T grows into the 1P peripheral nerve. Then a secondary branch from the posterior arm grows into the posterior interganglionic connective nerve, and the anterior arm grows into the anterior connective nerve. When Rs connective axons from adjacent segments meet, they fasciculate. Then a new axon grows from near the branch point of the T into the 1A peripheral nerve. Rs neurites which extend to the A ganglia form a complex network which extends from those in segments M5 and M6 enlarge to become the largest somata in a ganglia. Embryos of T. rubra were incubated for 2-3 days, during the period of axon outgrowth, in media containing 10 μM 5-HT or 10 μM ACH with 50 μM 5-HT. Rs morphology was visualized by anti-5-HT antibody staining or by fusaricine or anti-acetylcholine antibody staining. Following ACH incubation, initial axon outgrowth was normal. But when the connective axons of Rs neurons in adjacent ganglia would normally meet and fasciculate, all connective axons disappeared. A new anterior connective axon arose from the posterior arm 90-120° from the original anterior arm, and the MA axon arose from this new branch (instead of from the T). After injection of biocytin into the MA axon, Rs neurites regrew in the original posterior position. ACH incubation led to a substantial increase in the largest somata in Rs neurons, but not in other SHT neurons.

Prolonged 5-HT incubation reduced anti-5-HT staining in Rs neurons to near invisibility. Staining of other SHT neurons was unaffected. This selective effect on Rs reflects a change in transmitter interactions. In RS (Hirudo) (1b) incubation increases anti-5-HT staining intensity. Prolonged 5-HT exposure also caused all Rz somata to remain small, which is normal for segments M5 and M6. The results show that exogenous 5-HT or ACH can induce changes in transmitter content, soma size and axon branching in Rz neurons. Therefore Rs transmitter pairs are likely to play a role in the regulation of these decisions in Rz development.

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461.8 SHAPE CHANGES IN EMBRYONIC LEECH GROWTH CONES REFLECT TRANSITIONS IN OUTGROWTH. D. Kemp* and J. Jellis, Neurobiology Research Center and Dep. of Physiol. and Biophysics, Univ. of Alabama at Birmingham, Birmingham, AL 35294

Correlations between growth cone shapes and molecular and cellular guidance signals suggest that studying changes in morphology may help characterize these underlying guidance cues in an embryonic leech. We have analyzed the morphology of a set of growth cones on the Cumb- or C-cells in the embryonic medicinal leech, Hirudo medicinalis, as they undergo a transition to rapid outgrowth. The C-cell growth cone is a bipolar pair in each midbody segment. Each C-cell adds and orients about 70 parallel growth cones which remain relatively non-motile until E12 when rapid and directed process outgrowth is initiated. C-cell growth cones from the same segment at E10, E11, E12, and E14 were injected with Lucifer Yellow and processed with antibodies to visualize their growth cone in whole mounts. A central subset of ubiquitously oriented growth cones (n=583) were traced using IMC optics and camera lucida. Morphology was digitized from these tracings and a computer program (MacMeasure) was used for analysis and comparison. Contrary to expectations for growth cones that become more rapidly advancing, lamellae regions and filopodial sampling increased with age. Young, relatively non-motile growth cones had numerous short filopodia in many orientations, while the more rapidly advancing growth cones showed a decrease in filopodial number, an increase in filopodial length, and a striking restriction of filopodial orientation to the direction of process outgrowth. However, the average filopodial angle was predictive of the direction of outgrowth at all stages, suggesting that while direction of growth could always be predicted by a vector sum of filopodial trajectories, younger quiescent growth cones respond to different cues (or similar cues differently) than older, more rapidly extending ones. These results support the view that C-cell growth cones are fasciculating and further suggest that they progressively alter filopodial extension/integration in the manner predicted if their affinity for local cellular cues was superceded by a more distributed set of extrinsic guidance signals. Supported by NIH NS 26063 (JJ).

462.1 THE ROLE OF THE ZEBRAFISH SCLEROTOME IN PERIPHERAL NERVOUS SYSTEM SEGMENTATION. Elizabeth M. Morin-Kensicki* and Judith S. Eisen, Institute of Neuroscience, University of Oregon, Eugene, OR 97403

Sclerotome has been implicated in patterning peripheral nervous system (PNS) segmentation in vertebrate embryos. We have identified sclerotome cells in embryos of the zebrafish, Brachydanio rerio, based on the following criteria: these cells: 1) delaminate from the ventromedial aspect of each somite; 2) become mesenchymal; and 3) migrate to positions where vertebrae later form. In each embryonic zebrafish trunk segment, sclerotome cells migrate along a pathway that later is traversed by the growth cones of axial motoneurons and by some neural crest cells. We tested the role of sclerotome in patterning zebrafish dorsal root ganglia (DRGs) and axil motor nerves by ablating premigratory sclerotome by aspiration and observing the pattern of these PNS structures at later stages by staining with specific antibodies. Removal of sclerotome did not disrupt the pattern of the DRGs or the axial motor nerves. These findings suggest that zebrafish sclerotome is not required for proper formation and segmentation of DRGs or axil motor nerves. Our results are in marked contrast to findings in avian embryos where sclerotome does appear to be required for these processes and thus, we suggest that there are different mechanisms for establishing PNS segmentation in different vertebrate species.

NIH HD07348, HD22486, GM070257, NS32915

462.3 EXPANDED ROLE OF MIDLINE SIGNALING IN THE DORSO-VENTRAL BRAIN POLARITY OF ZEBRAFISH. Kota Hatsumi, Michael Westermann, and Charles B. Kimmel*, Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The zebrafish cyclops mutation deletes the entire ventral midline of the central nervous system (CNS), including the ventral forebrain and floor plate in trigeminal and spinal cord, without disturbing surrounding mesodermal structures. In the spinal cord and caudal hindbrain, positioning of most neurons is unaffected although pathfinding of some axons is severely impaired. The phenotype is more severe in the anterior CNS; in the diencephalon, the ventral neuronal groups and longitudinal axon bundles are deleted. To characterize the phenotype in more detail, we examined four homeobox genes (en, rhx3.3, pax2, pax6) that are expressed in specific positions in the CNS. Among them, pax2 is expressed in a wild type as well as in a null of domains in the diencephalic primordium just after gastrulation. These domains are fused at these stages in cyclops. At later stages, the mutant expression pattern of each of the homeobox genes shifts ventrally in more anterior regions of the brain. These results suggest that cyclops alters the early fate map and dorsal-ventral positional information of the CNS. Transplantation of a single wild-type cell that comes to occupy the ventral midline in the mutant locally rescues the forebrain and eye phenotypes. We argue that proper establishment of positional information in the midline is essential for both signaling from ventral midline cells, whose specification depends on the cyclops gene product. (Supported by NIH grants NS17903, HD22486, and HD22487.)


Using in situ hybridization and screening with homoeodomain-encoding probes, we isolated, from an E14.5 mouse telencephalon cDNA library, a 2.4 kb cDNA encoding a novel mouse homoeobox-containing protein called Tgs-1. The Tgs-1 homoeodomain is identical in 53 out of 61 amino acids with the homoeodomain of the Drosophila segmentation gene. Tgs-1 is part of the newly discovered Dlx family of homeobox-containing genes. It is also known as Dlx-2. Unlike other vertebrate homoeodomain-containing genes, Tgs-1 is predominantly expressed in the head. When ths a brain of the midgestational embryo, Tgs-1 is expressed in the ventral forebrain. In situ RNA hybridization shows that Tgs-1 is expressed in discrete domains within the midbrain/forebrain. In the diencephalon, these domains form sharp borders with the expression pattern of another homeobox gene, and a gene encoding a putative growth factor.

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PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING V

PATTERN FORMATION, COMPARTMENTS AND BOUNDARIES III

463.1 PATTERNING AND INDUCTION IN A ZEBRAFISH MUTANT THAT LACKS A NOTOCOTCH. M. E. Haber*, B. K Ho, S. Schulte-Merker*, C. Nusslein-Volhard*, C. Walker and C. B. Kimmel, Institute of Neuroscience, University of Oregon, Eugene, OR 97404, and Max-Planck-Institut für Entwicklungsbiologie, 72076 Tübingen, Germany.

In the zebrafish embryo, the notochord is derived from precursor cells that involute during gastrulation to form the notochord. As in other vertebrate embryos, dorsal mesoderm may play an important role in the induction of the central nervous system (CNS).

We have recovered a novel, recessive lethal mutation that produces embryos with no notochords or tails (ntt). Cell lineage studies suggest that dorsal mesoderm is present in ntt embryos, although subsequent notochord differentiation is blocked. Phenotypically, ntt embryos resemble mouse Brachyury (or T gene) mutants. They also fail to express a protein homologous to the mouse T protein, which is an early marker of notochord development. Molecular analysis confirms that nt is in fact the zebrafish homologue of the T gene.

Although the notochord is absent in nt mutants, the induction and differentiation of the CNS appears normal. In contrast, patterning of somitic mesoderm is abnormal due to the absence of endogenous signaling from notochord or pioneer cells. However, the somite defects can be rescued through transplantation of wild-type (WT) cells into ntt hosts. These experiments demonstrate that mutant host mesoderm is capable of forming muscle pioneers in the presence of notochord derived from WT donor cells.

We propose that the dorsal mesoderm has multiple cell signalling roles in the development of the zebrafish embryo. In ntt mutants, the signal required for CNS induction are retained, while the signals required for muscle pioneer induction and somite patterning are disrupted.

Segmentation has been implicated in the development of the spinal cord and hindbrain. In the forebrain, however, evidence for distinct embryonic domains—a hallmark of segmental development—has remained elusive. We report here that a distinct domain in the developing forebrain that is defined by early neurogenesis and specific axon ingrowth. This unique but transient domain appears in the outer 2/3 of the ventrolateral wall of the forebrain soon after the formation of two distinct telencephalic vesicles from the single prosencephalon. The earliest neurogenesis in the forebrain, indicated by AP-2 staining and DNA labeling, occurs in this zone. In addition, first neurites growing from the developing forebrain are limited to this region as shown by immunostaining for neurite specific molecules GAP-43 and L1 as well as by EM analysis. Oil-1 staining indicates that many of these neurites originate from the olfactory epithelium. The ventrolateral domain disappears as rudiments of the major forebrain subdivisions—the neocortex, hippocampus, basal ganglia, basal forebrain, and olfactory bulb—emerge.

This temporally and spatially restricted pattern of neurogenesis and neurite growth delineates a distinct domain in the developing mouse forebrain. This domain differentiates before rudiments of the forebrain subdivisions are seen and may be crucial to their formation. The ingrowth of axons from a specific source, the olfactory epithelium, further suggests that this regional specialization may be required for establishing initial axonal projections to the forebrain.

462.7 ACTIVATED RETINOIC ACID RECEPTORS DEFINE A DISTINCT DOMAIN IN THE DEVELOPING MOUSE FOREBRAIN. A.S. LaMantia*, A.K. McAllister, I.G. Whitesides, M. Colbert, and E. Linney. Departments of Neurobiology and Neuroimmunology, Duke University, Durham NC 27710.

The acquisition of regional identity in embryos is orchestrated by transcription factors acting within distinct domains. In the vertebrate CNS these domains have been seen in the developing spinal cord and hindbrain; however, they have not been described for the forebrain. We have shown a unique zone of early neurogenesis, differential cell adhesion, and neurite ingrowth from the olfactory placode in the developing mouse forebrain. We now show that the emergence of this zone is temporally and spatially correlated with that of a transcription factor, the retinoic acid receptor, using a transgenic detector mouse (Balkani et al, 1991:PNAS 88:3437-3451). The transgene contains three copies of the retinoic acid response element, a basal promoter, and 5'-galactosidase as the reporter gene.

At the prospechene stage, cells in the ventrolateral zone of the forebrain and the olfactory placode are transgene positive based upon anti-β-gal immunostaining. At the early telencephalic stage, transgene activity is coexpressed with neuron-specific markers. Neurons are seen in the outer 1/3 of the epithelium, while transgene activity is limited to proliferative cells in the middle 1/3. Similarly, transgene positive cells are segregated from neurons in the olfactory epithelium. Transgene activity decreases as more postmitotic neurons are observed in the ventrolateral zone and olfactory epithelium, and by the forebrain rudiment stage it is absent in both places. These observations support a role for region-specific transcriptional regulation in the earliest subdivision, differentiation and intervation of the mammalian forebrain. A.S. LaMantia is supported by a National Down Syndrome Society Scholastic Award.


Previously we identified an aldehyde dehydrogenase present at high levels in the dorsal retina of the embryonic and adult mouse as the basic isomerase AHD-2 known to oxidize retinaldehyde to retinoic acid (RA). Comparative estimates of RA levels with a reporter cell line placed the retina among the richest organs in the early embryo; levels in ventral retina, however, exceeded dorsal levels. A zymography biosassay, the test of charge-separated protein fractions for RA synthetic activity with the reporter cells, revealed a different, acidic dehydrogenase in ventral retina. Affinity purification shows a 60 kD protein whose presence and amount parallels the ventral enzyme activity. Per protein amounts, this putative novel enzyme is several hundred-fold more powerful in RA synthesis than AHD-2.

The relative contributions of the two enzymes to total RA synthesis in the eye region vary with developmental age. The acidic dehydrogenase precedes AHD-2; alone it mediates the very active RA synthesis we see in the optic grove and surrounding tissue at E8.5. At E10 the acidic enzyme accounts for ~90% of synthesis in the eye region, at E14 for ~70% of synthesis in the retina, and at E17 for ~50%. In the adult retina the acidic enzyme is no longer detectable and all RA synthesis is mediated by AHD-2. The patterns in the expression of the enzymes suggest (1) that the acidic dehydrogenase plays a role in the determination of the eye as an organ, and (2) a particular cell population, created by the use of different dehydrogenases, is specifically targeted at the biochemical basis of positional information.


Retinoic acid (RA) in the embryo is generated from retinaldehyde by a series of dehydrogenases that differ in spatial localization and enzymatic characteristics including inhibitor susceptibility. In the early mouse embryo the retina and the spinal cord have the highest RA concentrations. Synthesis of RA to the retina is mediated by retinaldehyde dehydrogenase, which is mediated by different dehydrogenases, one in the dorsal, the other in the ventral retina.

In tests of dehydrogenases inhibitors on RA-synthesizing enzymes in vitro and for teratogenic effects in vivo, we found several reagents with relatively selective effects. In the retina, dial sulfate depressed preferentially dorsal RA synthesis in all species tested. The retinal enzyme was much less susceptible to sulfinilamid; in the mouse it was relatively inhibited by p-hydroxymercuribenzoate. A combination of inhibitors to the monooxygenation stage of retinoid synthesis, the most striking effect was in the trunk region; after very low concentrations of the drug the trunk was shortened and the notochord appeared swollen and wavy; higher concentrations produced in addition other phenotypes, including visceral anomalies. Inhibition of RA synthesis may be involved in malformations linked to disulfinilamid (=Antabuse®) in experimental animals and humans.


Members of the engrailed (en) gene family encode highly conserved transcription factors, containing five conserved protein domains, encoding a homeodomain. The murine en genes, En-1 and En-2, are initially co-expressed in a spatially restricted pattern across the presumptive mid/hindbrain junction. Later, the genes are expressed in restricted groups of neurons important for motor control. To examine En-2 function, a mutation which deletes the homeobox of the En-2 gene was made by homologous recombination in embryonic stem cells. Mice homozygous for this mutation, En-2, exhibit a reduced cerbellar granular layer and a distinct patterning defect of the cerebellar folia. Examination of the developmental profile of foliation has revealed that at the midline, the secondary fissure occurs in a more anterior position, causing a fusion of the pyramis and the uvula. As well, laterally, the amonoparamedial fissure fails to form a fusion of Crus II with the paramedial lobe. Analysis of a lobe-specific lacZ transgene marker also indicates that a lobe transformation within the mutant cerebellum. In an attempt to identify genes that interact with En-2, we have begun mapping complex mutants with other mutants such as weaver and unc connect (that affect the cerebellum). In addition, we are testing whether the mouse homologs of genes such as wingless (wg) interact with En. In Drosophila, wg acts as a target for this interaction in mouse, Wnt-1, a homolog of wg, is expressed in a similar domain as the En genes during early development. Mice homozygous for a Wnt-1 targeted mutation exhibit a deletion of the mid-hindbrain region projecting to the brain. Preliminary genetic and morphological evidence suggesting that Wnt-1 interacts with En-2 in the patterning of cerebellar foliation. We are currently examining the expression patterns of Wnt-1, En-1 and En-2 genes during cerebellar foliation to correlate their expression with the observed phenotypes.
462.11
WHY DO WHISKER-RELATED PATTERNS FAIL TO DEVELOP IN THE BRAINSTEM AFTER FETAL NGF INJECTION?

We (Henderson et al., Neurosci. Abstr. 17, '91) have reported that rats given 20 or 30 μg of NGF systemically on embryonic day (E) 15 and again on E18 failed to develop whisker-related, cytochrome oxidase patterns in the trigeminal (V) brainstem nuclei by the time of birth. Controls indicated that this effect was not due to surgical trauma, reduced body weight, or reduced metabolic rate. In the present study, untreated rats given NGF daily for up to 3 postnatal days also failed to develop brainstem whisker patterns. A series of experiments were designed to test 3 hypotheses: 1) naturally occurring V ganglion cell death is ameliorated by NGF injections; 2) elevated NGF levels alters ganglion cell projections to the whiskerpad and/or 3) to the brainstem. Stereological analysis indicated that V ganglion cell numbers were higher than normal at birth after NGF injections at E15 and again at E18 (± 12% of control, p < 0.05) and brainstem whisker patterns did not develop. One injection at E15 did not alter cell numbers at birth (97 ± 12% of control, p > 0.05) and brainstem whisker patterns did develop. In cases where patterns failed to develop, Dil- and Dia-labeled infraratular nerve fibers had normal trajectories and projections to the whiskerpad were not unusual. Yet, Dilaabeled V primary afferent projections to the brainstem did not exhibit whisker-related patches. Thus, naturally occurring ganglion cell death may be important for the patterning of central primary afferent projections. DE97754, DE79662, NS24679.

NERVE GROWTH FACTOR VI

463.1

A transgenic mouse line has been obtained which carries the prepro-NGF gene under a c-fos promoter. The expression of the transgene in the brain and spinal cord has been assayed in vivo, in correlation with ELISA estimations of the amount of NGF synthesized and secreted by both cell types. In transgenic astrocytes, basal intra- or extracellular amounts of NGF were not significantly different from normal despite the presence of high amounts of mRNA specific for the transgene. Stimulation of the promoter with phorbol esters (TPA) produced a 10-fold increase in the secretion of NGF by both normal and transgenic astrocytes. Stimulation of the cAMP pathway by dibutyryl-cAMP or forskolin induced a 2- to 3-fold increase in the secretion of NGF. In transgenic neurons, intra- and extracellular basal levels of the protein were respectively 4- and 2-times above normal ones. Fetal calf serum, which increased by 4 the levels of NGF secreted by normal neurons had no effect on transgenic ones. TPA, which increased by 10 the amount of NGF secreted by normal neurons had a slighter effect on transgenic neurons while dibutyryl-cAMP and forskolin increased the secretion of NGF by 10 in transgenic neurons and by 3 in normal ones. The possibility to selectively regulate the production of NGF in transgenic embryonic tissue offers new insights to the in vitro studies of the factor and to in vivo investigations by transplantation.

463.2
DIFFERENTIAL INFLUENCE OF NERVE GROWTH FACTOR ON NEUROPEPTIDE EXPRESSION IN VIVO - A NOVEL ROLE IN PEPTIDE SUPPRESSION?

In normal adult rat, approx. 50% of lumbar DRG neurons have high-affinity receptors for NGF. A role for NGF in these neurons appears to be maintenance of differented phenotype as denoted by the ability of excess or NGF to counteract loss of cell body and CPG following injury. To examine further at a cellular level possible mechanisms underlying the role of NGF in regulation of peptide expression in intact and injured neurons, particularly the right sciatic nerve was transected two weeks prior to sacrifice. In other animals, NGF was infused intracerebrally (125μg/hr) for 8 days following the two-week insecetive period. Cytokine sections were processed for receptor radioautography with radiolabeled NGF and for in situ hybridization with 35S-labeled oligonucleotide probes to detect α-CGRP, β-CGRP, SP, SOM, VIP, CCK, NPY and galanin (GAL) mRNA. In normal neurons α-CGRP, β-CGRP, SP, and SOM are also expressed and are regulated by NGF; whereas few neurons express CCK, NPY and GAL. Immunohistochemistry and in situ hybridization confirmed that NGF receptors are widely distributed in the adult rat CNS. The injury-induced increases in VIP, CCK, NPY and GAL mRNAs. These results indicate NGF can act in vivo to maintain and possibly augment, the regulation of peptide expression in intact sensory neurons. We suggest that NGF may be part of the signal that allows reversion to normal of responses to injury when axons regenerate into the intact sciatic nerve conduit, a rich source of NGF. Supported by Canadian & Swedish MRCs.

463.4

Mouse trisomy 16 (Ts 16) is an animal model of Down syndrome (DS). We have previously shown that fetal Ts 16 brain fascia transplant undergoes time-dependent, cholinergic neuronal atrophy similar to that seen in DS and Alzheimer's disease (AD). We now asked whether the transplants contained NGF receptors and would respond to NGF. We transplanted cell suspensions of fetal Ts 16 or control fascia into the hippocampus of young adult mice. Six months later, mice received continuous infusion of either NGF or vehicle for 2 weeks. Grains were examined by immunohistochemistry or in-situ hybridization to characterize neuronal morphology and gene expression. In both Ts 16 and control grafts, β-CGRP and SP mRNA were expressed in cholinergic neurons and not in the host mouse hippocampus. Similar to prior findings, Ts 16 cholinergic neurons appeared atrophic and were significantly smaller than controls (β-CGRP, Tu < 0.001). The decrease in all cholinergic neurons (Ts 16: 201 μ² (208 ± 29 μ²), p < 0.001). There was no evidence of Aβ deposition. NGF reversed neuronal atrophy in an animal model of spontaneous, age-related, neurodegeneration. We speculate that NGF may be reversing a neuronal death program and that expression of candidate death gene can be examined in this model.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
NGF RECEPTOR ACTIVATION AND INTERNALIZATION IN PC12 CELLS.
Mark Grimm* and William C. Mobley
Department of Neurobiology, University of California, San Francisco, CA 94143.

Neurotrophins interact with receptors on neurites to cause a response in the distant cell body. To understand how the signal is carried to the cell body, we have studied early events in the intracellular movement of NGF in neurons undergoing neurite outgrowth. Radiolabeled NGF was cotransfected with the NGF receptor (p75 NGFR) into PC12 cells. After 18 h, NGF was internalized into a large fraction of NGF was released in ligand. Neuronal membrane structures that escaped when the NGF was reimmobilized to cell-free conditions. When the NGF was reimmobilized to cell-free conditions, it was observed that the NGF is released from the PC12 cells. We conclude that the NGF, when internalized into early endosomes, and that transport vesicles arising from early endosomes accumulate in vitro. Preliminary experiments suggest that both p75 NGFR and trk are present in these vesicles.

Nerve growth factor (NGF) induces sprouting of injured adult rat focal cholinergic neurons and promotes their regeneration into nerve grafts and hippocampal formation. We have investigated the potential neurotrophic or cholinergic effects of NGF in the adult rat focal cholinergic septohippocampal system. 1) Animals received bilateral sciatic nerve grafts between the disconnected septum and hippocampal formation. A 4-week NGF infusion into the rostral lateral ventricle caused sprouting of cholinergic fibers in the dorsal lateral septum, which extended toward the ventricle. 2) In mice with nerve grafts and 2-week infusions with NGF into the fornix (proximal to the lesion and grafts), axons sprouted in the medio-dorsal septum, i.e. toward the infused fornix. However, no fibers were seen in the nerve, i.e. regrowing axons had reached near the NGF source. 3) NGF infusion into the contralateral ventralis of animals with a unilateral fimbria-fornix transaction but no graft, induced sprouting toward the midline of the septum but not in the normal side. 4) Infusion of a low NGF dose into the lesioned septum induced sprouting only along the infusate. Thus, the location of an NGF source (the location of the highest NGF concentration) can control the orientation of outgrowing cholinergic axons, indicating that NGF has neurotrophic effects in the adult animal, as it has for developing peripheral nervous system in vivo and in vitro. Support: NINCDS grant NS-18549 and 27047.


The neurotrophic family of growth factors includes several neurotrophins, which are the most potent members of the growth factor (NGF), all of which promote neuronal survival and differentiation. The biological actions of neurotrophins are exerted via activation of tyrosine kinase receptors. In addition, all neurotrophins are recognized by a different receptor molecule, known as p75, which is required for NGF signal transduction and is expressed in neurons as well as in some non-neuronal tissues. In the preovulatory ovary, non-neuronal p75 expression is localized to theca cells of developing follicles. Immunochemical characterization of p75 in embryonic rat ovaries using a specific monoclonal antibody (PG1 190) revealed that the receptor is expressed in mesenchymal cells. By gestational day 18, these cells begin to form stroma "pockets" which, as gestation proceeds, separate the presumptive pre-granulosa cells into discrete groups surrounding individual oocytes. This enclosure continues postnataally resulting in an abrupt formation of primordial follicles between 24 and 48h after birth. In vitro exposure of neonatal ovaries in organ culture to affinity purified NGF antibodies (2.4µg/ml), resulted in a striking reduction (>80%) in follicular formation quantitated 48h later. Unexpectedly, this was accompanied by widespread cell death confined to p75-positive stromal cells. The results suggest that NGF and/or other members of the neurotrophin family are essential components of the differentiation program that governs follicular formation. (Supported by NIH grants HD24670, HD74748, HD18185, RR01663)
The Lck A, Lck B and Lck C genes have been shown to encode a class of receptors for nerve growth factor, a neurotrophic factor, neurotrophin 3 and neurotrophin 4. We have sought to study the role of these tyrosine kinase receptors in a vertebrate model of neurodevelopment, the zebrafish Brachydanio rerio. Nucleotide sequence analysis of 120 clones derived from P1 hybrids revealed five distinct representatives homologous to Lck A, Lck B and Lck C. A lambda ZAP cDNA library has been constructed from day 1 embryo RNA and cDNA clones have been isolated. Southern blot hybridization has been shown to show that each of these Lck sequences represent a unique zebrafish gene. Northern blot analysis reveals that these zebrafish Lck genes are expressed early in embryonic development. Some of the novel Lck members identified in zebrafish may be the receptors for previously uncharacterized neurotrophic factors. These results suggest that one or two Lck genes have not yet been described in mammals.

Intracerebral implantation of cells genetically altered to secrete high levels of NGF has profound effects on local neuronal survival after excitotoxic or knife-cut lesions. The effects of such biological NGF delivery systems, specifically the induction of genes encoding protective enzymes or stress proteins in surrounding neurons or glia, has not been well studied. We chose to investigate the effects of an implanted NGF-secreting fibroblast cell line on local levels of acetylcholinesterase (AChE), the stress proteins c-fos, c-myc, heat shock protein-72 kDa (hsp72), and ubiquitin (UB), and the peroxidase enzyme catalase. Seven days after unilateral corpus callosum implantation of either a genetically altered NGF-producing (NGF+) or control (NGF-) fibroblast cell line, we stained tissue sections surrounding either the NGF+ or NGF- grafts using antibodies raised against c-fos, c-myc, or Ub, though both types of grafts stained faintly for c-fos. Both NGF+ and NGF- grafts induced a high level of hsp72 immunoreactivity in tissue immediately surrounding the grafts. AChE staining was unchanged in areas adjacent to the grafts, and though there was no fibroblast AChE staining, multiple AChE positive fibers appeared to be growing into or traversing the NGF+ grafts. In contrast to the above, NGF+ cells caused increased catalase staining in astroglial cells adjacent to the grafts. These catalase positive astroglial cells were not seen in areas distant from the NGF+ grafts, nor were they seen in brains implanted with NGF- cells. These results suggest that the local protective effects of NGF+ fibroblast grafts may be mediated through changes in peroxidative metabolism due to increased levels of catalase. If so, NGF-mediated protection and neurotropism in the adult brain may be caused in part by an increase in cell resistance to lipid peroxidation.

**Molecular Cloning and Developmental Analysis of Five Lck Genes in Zebrafish.**

S.C. Martin*, J.W. Wo and G. Heinrich

University Hospital and Boston University School of Medicine, Boston, MA. 02118

NGF has not been well studied. We chose to investigate the effects of an implanted NGF-secreting fibroblast cell line on local levels of acetylcholinesterase (AChE), the stress proteins c-fos, c-myc, heat shock protein-72 kDa (hsp72), and ubiquitin (UB), and the peroxidase enzyme catalase. Seven days after unilateral corpus callosum implantation of either a genetically altered NGF-producing (NGF+) or control (NGF-) fibroblast cell line, we stained tissue sections surrounding either the NGF+ or NGF- grafts using antibodies raised against c-fos, c-myc, or Ub, though both types of grafts stained faintly for c-fos. Both NGF+ and NGF- grafts induced a high level of hsp72 immunoreactivity in tissue immediately surrounding the grafts. AChE staining was unchanged in areas adjacent to the grafts, and though there was no fibroblast AChE staining, multiple AChE positive fibers appeared to be growing into or traversing the NGF+ grafts. In contrast to the above, NGF+ cells caused increased catalase staining in astroglial cells adjacent to the grafts. These catalase positive astroglial cells were not seen in areas distant from the NGF+ grafts, nor were they seen in brains implanted with NGF- cells. These results suggest that the local protective effects of NGF+ fibroblast grafts may be mediated through changes in peroxidative metabolism due to increased levels of catalase. If so, NGF-mediated protection and neurotropism in the adult brain may be caused in part by an increase in cell resistance to lipid peroxidation.

**Immunological and Ultrastructural Localization of Low- and High-Affinity NGF Receptors on Neuronal Cells.**

P. Spoerri*, L. Rettelli, E. Dal Toso and S.D. Shapira

Fidia Research Laboratories, Abano Terme, Italy.

Responsiveness of neural cells to NGF appears to require expression and ligand binding to both the low-affinity NGF receptor (LNGFR) and the protooxygenase product trk, the latter being a receptor tyrosine kinase. We recently described the immunolocalization of LNGFR on PCL2 phaeochromocytoma and C6 glioma cells using immunogold electron microscopy. We now demonstrate the immunolocalization of LNGFR and the high-affinity component of the NGF receptor, trk (HNGFR), on the former cells and cultured neonatal rat dorsal root ganglia neurons using a double labeling technique. Receptor-specific antibodies were utilized in conjunction with immunogold conjugated to colloidal gold particles of different sizes. NGF-treated cells displayed considerable colocalization of LNGFR-HNGFR immunoreactivity (IR). Gold particles associated with LNGFR were by far the more numerous, being frequently seen near 2-3 (or more) gold particles delineating the HNGFR. Positive trk-IR thus seems to colocalize with LNGFRs in at least these neural cells.

**Local Response to Intracerebral Grafts of NGF-Producing Fibroblasts: Induction of a Peroxidase Enzyme.**

D. M. Prim, I. Schuhmacher, M.P. Ston, J.O. Brooksfield and D. L. Jansen

Neuroregeneration Laboratory, McLean Hospital, Belmont, MA.; Molecular Neurogenetics Unit, Neurology and Neurosurgery Services, Massachusetts General Hospital, Boston, MA.

NGF has not been well studied. We chose to investigate the effects of an implanted NGF-secreting fibroblast cell line on local levels of acetylcholinesterase (AChE), the stress proteins c-fos, c-myc, heat shock protein-72 kDa (hsp72), and ubiquitin (UB), and the peroxidase enzyme catalase. Seven days after unilateral corpus callosum implantation of either a genetically altered NGF-producing (NGF+) or control (NGF-) fibroblast cell line, we stained tissue sections surrounding either the NGF+ or NGF- grafts using antibodies raised against c-fos, c-myc, or Ub, though both types of grafts stained faintly for c-fos. Both NGF+ and NGF- grafts induced a high level of hsp72 immunoreactivity in tissue immediately surrounding the grafts. AChE staining was unchanged in areas adjacent to the grafts, and though there was no fibroblast AChE staining, multiple AChE positive fibers appeared to be growing into or traversing the NGF+ grafts. In contrast to the above, NGF+ cells caused increased catalase staining in astroglial cells adjacent to the grafts. These catalase positive astroglial cells were not seen in areas distant from the NGF+ grafts, nor were they seen in brains implanted with NGF- cells. These results suggest that the local protective effects of NGF+ fibroblast grafts may be mediated through changes in peroxidative metabolism due to increased levels of catalase. If so, NGF-mediated protection and neurotropism in the adult brain may be caused in part by an increase in cell resistance to lipid peroxidation.

**Reversible Abolition of Visual Motion Processing Mechanisms in the Lateral Suprasylvian Cortex of the Behaving Cat.**

S.G. Lomberg, P. Comwell, J.S. Sun, M.A. MacNeil and B.R. Payne

Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA. 02118

The purpose of the present study was to test the hypothesis that the lateral suprasylvian (LS) visual cortex of the cat is critical for the identification of a stationary pattern partially obscured by a moving mask. Two cats were trained to perform a task involving the discrimination of different shapes. The task was performed with and without the moving mask. The results showed that the LS cortex was critical for the extraction of stationary patterns from a field filled with contours in motion. The absence of LS cortical activity this ability is abolished. (Supported by NIMH #4647 and the Comwell Family).
464.3 INTEGRATING VISUAL MOTION RESPONSES FROM NEURONS IN COR-TICAL AREA MT BY ADAPTIVE FILTER SELECTION. S. J. Newhall* and T. J. Siwczyk. The Salk Institute, La Jolla, CA, 92037.

Integrating the responses from multiple motion-sensitive neurons in the visual cortex of the monkey may improve motion perception. We have developed an algorithm that combines the responses of multiple motion-sensitive neurons in the MT cortex of the monkey to generate a single output signal that is more reliable than any of the individual neurons.

464.4 CORRELATED ACTIVITY OF NEURONS IN AREA MT. E. Zohary*, M. N. Shadlen, and W. T. Newsome. Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

We have found that the activity of neurons in MT is correlated with the activity of other neurons in the same or different areas. This correlation is not due to chance, as the correlation is stronger when the neurons are in the same area, and it is weaker when the neurons are in different areas. The correlation is also stronger when the neurons are in the same layer, and it is weaker when the neurons are in different layers.

464.5 PREDICTING PSYCHO PHYSICAL PERFORMANCE FROM POOLED NEURONAL RESPONSES. M.N. Shadlen*, W.T. Newsome, K.H. Breen, E. Zohary and J.A. Movshon*. Dept. of Neurobiology, Stanford University, Stanford, CA 94305; Howard Hughes Medical Institute and Center for Neural Science, New York University, New York, NY 10003.

We have developed a model that predicts the performance of a population of neurons based on the activity of a single neuron. The model is based on the idea that the activity of a single neuron is a good predictor of the performance of the population because the activity of the single neuron is a good predictor of the performance of the population. The model is tested on data from monkeys performing a visual discrimination task, and it is found to be accurate.

464.6 RESPONSES OF NEURONS IN AREA MST DURING DIRECTION DISCRIMINATION PERFORMANCE: A COMPARISON OF NEUROLOGICAL AND PSYCHOPHYSICAL SENSITIVITY. Simone Ceolini and W.T. Newsome*. Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

We have found that the activity of neurons in MST is correlated with the activity of other neurons in the same or different areas. This correlation is not due to chance, as the correlation is stronger when the neurons are in the same area, and it is weaker when the neurons are in different areas. The correlation is also stronger when the neurons are in the same layer, and it is weaker when the neurons are in different layers.
464.10


Extraocular visual auras are involved in motion vision. Focal tMS of striate cortex degrades spatial acuity for letter trigrams and Landolt-C stimuli. TMS of extrastriate cortex may also degrade spatial acuity for random dot stimuli. Subjects identified the direction that correlated dots moved, using a background located 10 deg to the left or right of fixation. Psychometric functions for this four-direction, forced-choice task were fitted. The percentage of correlated dots required to achieve 80-90% correct responses was determined. The test was then repeated with additional randomly interspersed trials at the determined percentage of correlated dots during which TMS was applied 50-250 msec after the onset of the 50 msec motion stimulus. TMS was applied with a 9 cm coil over the lateral posterior hemispheres at an intensity of 1.8 Tesla. In five subjects, TMS degraded the motion perception when applied 100-150 msec after stimulus onset. TMS produced a 36% mean decrease (range 33% to 44%) in correct responses when applied 125 msec after motion stimulus onset. In three subjects, little or no decrease was found when TMS was applied 50-75 msec or 175-250 msec after stimulus onset. This study was repeated over human extrastriate cortex transiently degraded motion perception and may be valuable in tracing pathways of visual information processing. (Supported by NEI E03387 and The Valley Foundation).

464.11


Transcranial magnetic stimulation (TMS) in healthy human subjects (N=42) can produce specific reversible defects of visual perception. Single TMS pulses are applied systematically to different locations over the central, temporal, parietal and frontal cortex using a Cadwell M10-10 magnetic stimulator device with a maximum output of 2 Tesla peak flux density. A number of different visual tasks have been employed. In a task with visual letters, the delay between stimulus onset and TMS, and type of magnetic coil have all been varied. In the motion perception task, for instance, a probe with a 14x14 dot matrix (0.01° x 0.01°) oriented parallel to the short term memory scanning, motion direction detection and color identification. The effects of visual stimulation (200 Hz) were studied on the visual evoked potential (VEP). The obtained VEP amplitude at the brain central level was measured.

464.12

Electrophysiological Concomitants of Apparent Motion in Man. R. Pfeffermann, V.G. Seminotti. Center for Neuroactive Drugs, Dept. of Motion Analysis, Institute of Neurosciences, Salk Institute, La Jolla, CA 92037; Institute of Cybernetics and Biophysics, CNR, Genova, 16132 Italy.

A Witheiser's paradigm is used to induce the visual perception of apparent motion was approximated on a VENUS Neuroscientific system by producing a bidimensional 5x5.9 deg image of alternating black and white sinusoidal vertical bars counterphasing at 0.5-8 Hz. Stimulation was at 0.4 or 5.0 cd/lm (or temporal frequency parameters used to produce (visual) retention and cortical evoked potentials in man. The signal was recorded 1.00.00.0 HI via dermal electrodes located on inferior eyelid (reference: contralateral upper and occipital area; ground; midfrontal), and processed offline (512 sample/second) by FFT. Eight healthy subjects were recorded; all agreed in reporting the expected perception of apparent motion with high probability at 2.0 Hz or 5.0-8.0 Hz temporal frequencies with visual evoked potentials at 10.0 and 5.0-5.0 cd/lm, respectively. Significantly higher amplitudes of the visual and cortical 2nd harmonic were observed at these temporal frequencies compared to higher or lower frequencies (512 Hz) when the percept was not reported to be as evident and the latency amplitude was higher than 2nd harmonic. The observation was consistent between and within subject, and suggests a potential concurrence of Witheiser's phenomenon with the spatial/temporal frequency-dependent functions of the visual system concurring in the generation of retention/cortical visual evoked potentials.

465.1

'H and 3P in Vitro NMR Studies of Chronic Hypoxia in FISHER 344 RATS. R. Kalonym* E. G. N. Exner, 3P. Lab of Neurology, University of Pittsburgh, Pittsburgh, PA 15261

Recent biochemical and clinical observations suggest that repeated, brief, mild energetic stress could, in some individuals, trigger molecular and metabolic mechanisms that result in the biochemical findings in the brains of Alzheimer disease (AD) patients. In this study, we investigated the biochemical responses of Fischer 344 rat brains by subjecting them to repeated hypoxia for 30 seconds for a period of two weeks. Three months and 10 days old animals were used to investigate age-dependent changes in metabolic changes under hypoxic stress. Of 3P NMR studies of the free clamped brains of controls and affected animals at the end of the two weeks indicate: (1) a nonsignificant increase in the level of the excitatory amino acid aspartate and glutamate in 3 month old animals. In the 10 days old animals, aspartate level was unchanged, whereas a 5% elevation (p=0.07) of glutamate was measured; (2) the inhibitory amino acid GABA is increased by 20% in 3 month old animals (p=0.04) and unchanged in 10 day old animals; and, 3P levels were decreased by about 9% in 3 month old animals (p=0.02) and by about 4% in 10 day old animals. These preliminary results indicate that aged animals are more susceptible to energetic stress than the young animals. Our 'H NMR results indicate a permanent alteration in some amino, 3P levels but not in others. However, 3P NMR results show no significant alteration in energy metabolism or membrane phospholipid metabolism indicating recovery of the brain from those energetic stress.

465.2

Glutathione Depletion Induces Heme Oxygenase-1 (Hsop2) mRNA and Protein in Rat Brain. J. P. Everin and M. D. Malins. Dept. of Biophysics, Univ. Rochester School of Medicine, Rochester, NY 14621

In mammals, heme oxygenase isoforms, HO-1 and HO-2, cleave heme to produce bile pigments. HO-1 is a stress protein (HSPO2) induced by chemicals in systemic organs the sensitivity of the rat brain. HO-1 mRNA and protein to cellular glutathione (GS) levels and provides the first evidence of reciprocal regulation of two antioxidant pigments: GSH and bile pigments in the rat brain. The treatment of rat brain with diethyl malate (DEM) (4.7 mmol/kg ip) caused a pronounced decrease in brain GSH levels. GS levels remained depressed for at least 24 hr post-injection. Northern blot analysis of DEM treated brain revealed an increase in HO-1.1-1.6 Kb mRNA up to 10-fold that of controls in a manner reciprocal to that of GS. Similarly, treatment of neonatal rats with buthionine sulfoximine (BSO, 0.5 mmol/kg ip; twice daily, 2-2 days of life) caused a marked decrease in brain GSH and a concomitant 10-fold increase in brain 1.6 Kb HO-1 mRNA above control levels. In contrast, the level of two homologous HO-2 transcripts (1.3 and 1.9 Kb) did not increase in response to either DEM or BSO treatment. Analysis of brain HO-1 immunoreactive protein following in DEM treated induced HO-1 protein in only select non-neuronal cell populations. Furthermore, the epidymal cell line expressing these proteins, Bergmann glia, cerebellum, leptomeninges lining brain and glia throughout brain responded to treatment by increasing HO-1-like immunoreactive elements.

We suggest that when glutathione induces HO-1 protein, hence increased capacity to form bile pigments, may be vital to those brain cells which normally depend on the triplepeptide for antioxidant defense. Supported by NIH Grants R37 ES03491, ES03968 and ES01247.

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Dept. of Biology, UCLA, Los Angeles, CA 90024. 

Catalytic, pharmacological and molecular studies have recently identified the genetically variable cytochrome P450IDI in mammalian brain. P450IDI (debrisoquine hydroxylation) is responsible for the metabolism of many drugs including neuroleptics, codeine and antidepressants. In addition, significant overlap was observed between substrates inhibitors for the dopamine transporter and for the P450IDI, for example, (-)cocaine, MPTP, methylenidate and d-amphetamine. In order to study this CNS P450IDI, we used PCR to detect the presence of P450IDI and cell marker RNA in immortalized cell lines. 10 of 15 centrally-derived cell lines were positive for the P450IDI RNA. Variation of RNA levels in 8 rat brain regions was also studied. In addition, 2 of 3 pancreatic cell lines and a fibroblast cell line also contained the P450IDI RNA. Studies of regulation, inducibility and enzyme activity are underway. Cell lines containing P450IDI should enable us to further our understanding of the role of the P450IDI enzyme in the CNS. (RFT is supported by the MRC of Canada)


The etiology of tardive dyskinesia (TD), a frequent and usually irreversible side effect of the chronic use of neuroleptics, remains unknown. Because available pathologic data do not support presynaptic hypotheses and implicate in the mitochondrial electron transport chain have recently been documented in several movement disorders, we thought it important to test the possibility that neuroleptics may affect mitochondrial respiration. Accordingly, adult rats received fluoxetine (Flu; 4 mg/kg) in saline daily for 30 days and were sacrificed 1.4 and 7 days after the last injection. Control animals received vehicle only. Chronic Flu administration caused significant changes in complex I, II, III and IV activities in both caudate-putamen and nucleus accumbens compared to controls. The effect of Flu on mitochondrial respiration was partially reversed as stratal complex I activity improved 7 days after the last injection, while the other complexes still exhibited some alterations. In addition, brain mitochondria prepared from naive rats and incubated with different classes of neuroleptics showed reduced complex I activity as compared to controls. Our findings with neuroleptics in rats may be relevant to the underlying pathophysiological mechanisms of TD.

465.7 *Kainate Receptor Expression Induces Toxicity in Fibroblasts and Hippocampal Slice Cultures.* L. Bergold, J. Casaccia-Bonnefil, and R. Pederson. SUNY-HSCB, Brooklyn, NY, and 4 The Albert Einstein School of Medicine, Bronx, NY. 

The CA3 region of the hippocampus is prone to damage by the excitotoxic kainate (KA) through potential mechanisms involving either recurrent excitation and/or the high expression of KA receptors. In order to develop a gene transfer system to study KA toxicity, a cDNA encoding the KA receptor subunit, gluR6, (provided by S. Heineman) was cloned into a defective HSV:1 viral vector, and used to transfer and express KA receptors in NIH3T3 fibroblasts and organotypic slice cultures. Fibroblasts infected with HSVgR6 became sensitive to KA toxicity, whereas infection with HSVlac, which expresses g-glutamidas, did not. This suggested that HSVgR6 efficiently transferred and directed expression of KA receptors, resulting in toxicity after exposure to KA. Slice cultures were infected by a 250ml microinjection of virus to the stratum pyramidale of CA1 or CA3. Infection with HSVlac resulted in expression of the reporter gene in 11-13 cells centered around the stratum pyramidale with no overt damage to the slice culture. In contrast, microinjection of HSVgR6 to CA1 and CA3 produced target site effect. The effect was limited to the site of injection, most CA1 pyramidal cells were killed, suggesting that recurrent excitation in CA3 may be an important transsynaptic excitotoxic mechanism.

465.8 *Excitotoxic Lesions in Organotypic Hippocampal Slice Cultures.* P. Casaccia-Bonnefil* and P. Bergold.4 Program of Anatomy and Cell Biology and Department of Pharmacology SUNY-HSCB, Brooklyn, NY 11203. 

Slow neuronal excitotoxicity is believed to underlie many chronic neurodegenerative diseases. A 36 hour treatment of organotypic hippocampal slice cultures in low concentrations of glutamate agonists or GABA-A antagonists results in loss of specific populations of both principal cells and interneurons three days after the drugs are removed. The loss of principal cells is detected by methylene blue staining, while subpopulations of interneurons are detected with anti-neuronal antibodies. GABA-A antagonists as well as calbindin D28K, parvalbumin, neuropeptide Y, somatostatin, and VIP. A complete loss of dentate granule cells and most hilar neurons is seen after treatment with 50μM glutamate. This treatment has no effect on CA1 or CA3. Application of concentrations of picROTOX up to 500μM does not appreciably increase the lesion. Exposure to 50μM kainate kills only CA3 piriform cells and hilar cells, and 10μM NMDA kills only CA1 neurons. Exposure to 1μM of either agonist has no effect. Co-application of both 50μM picROTOX and 1μM kainate, in contrast, destroys CA3, the hilus and the dentate gyrus. This suggests that specific patterns of delayed excitotoxicity can result from the synergistic action of both increased excitation and decreased inhibition.
465.9 CALPAIN INHIBITORS PREVENT CAPSAICIN-DEPENDENT NEUROTOXICITY IN DORSAL ROOT GANGLION NEURONS. P.S. Chordi1, J.R. Savidis, D. Bleasman & R.L. Miller, Dept. Pharmacol. and Physiol., Univ. of Chicago, Chicago, IL 60637.

Capsaicin (N-methyl-N-vanillyl-6-nonalamide) exerts a specific excitatory action on nociceptive sensory neurons resulting in elevated (Ca²⁺), and subsequent neuronal degeneration. A postsynaptic depolarizing current (iCa²⁺) with cell death is the Ca²⁺-dependent thiol-protease, calpain. In the present study we used cultured dorsal root ganglion (DRG) neurons. Inhibitors can prevent capsacain-mediated neurotoxicity. DRG neurons were isolated from 3-5 day old rat pups and grown in culture for up to 35 days. Two functional assays were employed to determine the number of capsacain sensitive neurons in the culture: (1) Calpain stimulated Ca²⁺-uptake; after exposure to 1-10μM capsacain for 20 minutes, in the presence of 5μM Ca²⁺, silver nitrate revealed that approximately 30-40% of neurons examined were sensitive. (2) Fura-2 based microfluorimetry; bath application of capsacain (1-10μM for 30-60 seconds) resulted in increases in [Ca²⁺] in 30-45% of DRG neurons examined. The capsacain response in both (1) and (2) were prevented by applying the capsaicin receptor antagonist ruthenium red (1μM), but were unaffected by the thiol-protease inhibitors E64 (10μM) or the calpain inhibitor MDL-18170 (10μM). Capsacain-mediated neurotoxicity was assessed by adding capsacain (1-10μM) to DRG cultures for 20 minutes in 5μM or 10μM extracellular Ca²⁺ ([Ca²⁺]o) and after 17-24 hours using propidium iodide/fluorescein diacetate fluorescence to determine the percentage cell survival. Capsacain treatment (30-100μM) in 10μM Ca²⁺, produced cell death in up to 30% of neurons. However, when either E64 or MDL-18170 (both at 10μM) were added to the media, the death produced by capsacain treatment was completely prevented in 5μM Ca²⁺ (p<0.001, n=4) and partially prevented in 10μM Ca²⁺ (p<0.001, n=4). [Ca²⁺]o (1-10μM extracellular Ca²⁺), preincubation with the Ca²⁺-chelator BAPTA-AM (50μM) or addition of ruthenium red (1μM) also prevented capsacain-dependent cell death. These experiments provide strong evidence that capsacain neurotoxicity is mediated via activation of the Ca²⁺-activated protease, calpain.


Forty rabbits were given either vehicle or ddc, by oral intubation, at a dose of 35 mg/kg/day for 24 weeks. The sciatic nerves were examined at 4 weeks intervals beginning at 8 weeks. Myelins splitting at the interperiodic line and intramyelinic edema were first evident at 16 weeks and closely correlated with enlarged mitochondria with abnormal ultrastructure. Analysis of a myelin fraction isolated from the nerves showed that ddc treatment did not affect protein distribution, lipid distribution or CNPase activity. No abnormalities were noted in neurons of dorsal root ganglia. We conclude that the primary effect of ddc is on mitochondrial function in Schwann cells with subsequent myelin degeneration.


The accessory radula closer muscle (ARC) and its innervation provide a model system for studying the role of neuromodulation and co-transmission. The ARC is innervated by two cholinergic neurons, B15 and B16, and a serotonergic modulatory neuron, the MCC. The motorneurons also contain modulatory peptide co-transmitters falling into four families. B15 contains the SCPs, buccalins, and FRPs, and B16 contains the buccalins and myomodulins. We have developed a method using RIA to directly measure SCP and buccalin release from the ARC following intracellular stimulation of a single motorneuron. We previously showed that SCP and buccalin are stored in the same dense core vesicles, and co-released in response to pharmacologically relevant patterns of B15 stimulation. Here, we report the effects of some of the modulators on peptide release from B15. Buccalin A, at 5×10⁻⁷M, decreased SCP release from B15 suggesting that buccalin released from B15 terminals can inhibit its own release, since SCP and buccalin are co-released. Furthermore, buccalin released from B15 may decrease the sensitivity of B15 because stimulation of the two motorneurons together produced less SCP release from B15 than stimulating B15 by itself. SCPs, at 10⁻⁷M, had no effect on buccalin release from B15, suggesting that SCP release from B15 does not affect buccalin release. Serotonin, at 5×10⁻⁷M produced an increase in SCP release from B15, as did stimulation of the MCC. These results suggest that the extrinsic serotonergic modulatory system can affect the intrinsic peptidergic modulatory system and that the intrinsic modulatory system can affect itself.


The peptides SCP and myomodulin (MM) are contained in the ARC motor neurons B15 and B16 and increase ARC contraction amplitude and relaxation rate. The mechanism by which these changes occur was investigated by SDS-PAGE and protein phosphorylation and phosphatase assays. We previously reported that synthetic peptide application to intact ARC muscle induced phosphorylation of a ±500 KDa protein. B15 neurons were stimulated intracellularly with individual current pulses at physiological rates for 10 min. Stimulated and non-stimulated muscle homogenates were phosphorylated using a back-phosphorylation paradigm with γ[³²P]ATP in the presence and absence of CAMP. Protein stimulation and the expression of the 70 kDa CAMP induced incorporation of [³²P] into a ±500 KDa protein in ARC muscle. Stimulation of B16 at physiological rates for 10 min also prevents CAMP induced incorporation of [³²P] into this protein. These results indicate that either B15 or B16 stimulation induces phosphorylation of this band. The phosphorylation could be due to either increased kinase activity or decreased phosphatase activity. Measurements of phosphatase activity following MM application show that phosphatase activity is not changed. Direct evidence implicating PKA in the phosphorylation of this band was provided by forward phosphorylation experiments in which muscle homogenates containing either SCP or MYO were treated with a specific peptide PKA inhibitor (P1K). P1K inhibited the phosphorylation of the ±500 KDa protein in a dose dependent manner. These results suggest that SCP and MM conjugate on the same substrate protein and utilize the same second messenger pathway.
**466.3** EFFECTS OF NEURON B16 STIMULATION AND MYODUOULIN APPLICATION ON cAMP AND PKA LEVELS IN ARC MUSCLE OF APISIA. S.L. Hooper, E.C. Cropper, W.C. Probst, I. Kuentzmann & K.-R. Wess. West Dept Physiology, M. Sinai Sch Med, NY, NY 10029. Ctr. Neurobiol. & Behav., Columbia U and NY Psych Inst, NY, NY 10023. The SCPs and myodoulins (MMs), modularly neuropetides present in the intestine of the ARC, exert similar action on ARC contraction amplitude and relaxation rate. The peak SCPs action at the PKA target site transduction pathway. Our studies (1990) on phosphodiesterase data (Probst et al., this volume) suggest that MM may also utilize cAMP and PKA as a second messenger system.

We now report that cAMP application to ARC muscles causes dose dependent cAMP increases, but to maximal levels only approximately one tenth that induced by SCP application. The maximum increases of cAMP levels following physiologically relevant stimulations of neurons B5 (SCP) and B16 (MM) are approximately the same. However, unlike SCP application or B15 stimulation, MM application or B16 stimulation show desensitization, with cAMP levels decreasing rapidly with continued B16 stimulation or MM application.

Unlike SCP, MM application to intact ARC muscles induces only small increases of active PKA in low speed super centrified muscle homogenates. However, MM induces large increases (though still small compared to those induced by SCP) in active PKA in both uncentrifuged homogenates and in resuspensions of the pellet fraction, suggesting that MM induces both PKA activation and translocation to the pellet. Taken together, these data indicate MM'S effects are likely mediated via the cAMP/PKA signal transduction pathway. The rapid dose dependent desensitization of MM induced cAMP augmentation may contribute to the depression of ARC contraction amplitude seen when high doses of MM act on the ARC.

**466.5** PEPTIDE IMMUNOREACTIVITY IN IDENTIFIED SENSORY AND MOTOR NEURONS OF MEDICAL LEECH EMBRYOS AND ADULTS. J.W. Bledsoe, I. Sellen and M.P. Nathanson. Neurobiology Research Center and Department of Physiology & Biophysics, Univ of Alabama at Birmingham, Birmingham, AL 35294.

Identified neurons in the medicinal leech, Hirudo medicinalis, are known to express several neuropeptides. Using an antibody against a synthetic peptide, crinacine pigmented dermopa (PDH) (Dirksen et al., Cell Tissue Res. 250:377, 1985), we have observed the PDH nerve cord for innervation. In the adult, the anti-PDH labels a large number of neurons in both the ventral and dorsal aspects of each ganglion. We used electrophysiological techniques and microinjection of the fluorescent dye Lucifer Yellow to identify some of the neurons and then double-labelled the preparations with anti-PDH, visualized with a rhodamine-conjugated secondary antibody. Of particular interest was the double-label of two well studied neurons, including a primary sensory neuron known as the lateral encapsope (NE) cell and the heat-activated (HE) motor neuron. There has been no previous data to suggest possible neurotransmitters or modulators for any of the primary sensory neurons. In contrast, the HE was previously shown to be cholinergic and to also express a FMRFamide epitope (Kuhlman et al., J. Neurosci. 5:2301, 1985). Certain other FMRFamide labeled neurons, including the dorsal excitatory motor neuron 3, showed no staining with anti-PDH. Other double labeled neurons include the nitropeptide and the inhibitory motor neurons 1 and 2. In embryonic Hirudo, the N-cell appears to be the first neuron labeled by anti-PDH, being distinguished by embryonic day 10 (E10). The HEs are identifiable using this antibody by E12, which is 3-4 days prior to the earlier FMRFamide labeling. Additionally, a cell that has been identified as a possible HE homolog in ganglion 2 labeled at the same stage of development as the definitive HEs. This staining pattern and temporal progression argue that the PDH-epitope is carried by a different antigen than that which precipitates a FMRFamide epitope. Supported by NSB3606D(U) and NS39463(MPN).

**466.6** OCTOPAMINE-IMMUNOREACTIVE NEURONS IN THE LOBSTER CNS. H. Schneider, B.A. Trimmer, J. Rapus, M. Eckert & E.A. Kravin*. Harvard Medical School, Neurobiology Dept., Boston, MA 02115, and University Jena, Tierphysiologie, 07580 Jena, FRG.

With an antibody directed against an octopamine-gluteraldehydethioglycol complex we detected about 86 immunoreactive neurons within the entire CNS of 4th stage larval of the American lobster, Homarus americanus. The cells are distributed as follows: brain - 12, circumoesophageal ganglia - 2, suboesophageal ganglia - 38, thoracic ganglia - 6 each, and abdominal 4th and 5th abdominal ganglia - 2 each. All the octopamine-immunoreactive cells are paired and located along the dorsal or ventral midline. Of the 86 neurons, 24 have been identified as neurosecretory cells, and 32 are intersegmental ascending thoracic, ascending abdominal, or descending interneurons. The neurosecretory system is arranged segmentally and located entirely within the thoracic and suboesophageal ganglia. The projections of these neurons elaborate extensive varicose fibres along the proximal regions of 2nd thoracic and suboesophageal roots. These neurons are complementary to 2 pairs of large serotonin-containing neurosecretory neurons found in the fifth thoracic and first abdominal ganglia. The sets of neurosecretory neurons are arranged differently: the serotonin cells are intersegmental while the octopamine cells are segmental. Using a biochemical assay, the cell bodies of octopamine-immunoreactive neurosecretory cells in the thoracic segment of the nerve cord were found to contain 40-100 fmol of octopamine, while control neurons had none. Supported by NHI and DFG Sch 568/1-1.

**466.7** IDENTIFICATION OF A DROSOPHILA HISTIDINE DECARBOXYLASE GENE REQUIRED FOR PHOTORECEPTOR SYNAPTIC TRANSMISSION. J.Y. Berkey*, R.J. M. Solek, D.W. Ch. Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN and Dept. of Ophthalmology, Northwestern Univ. Med. School, Chicago, IL.

Histamine has been proposed to be the synaptic transmitter used by interneuronal photoreceptors, including Drosophila. Using genetic and molecular cloning approaches, we identified a Drosophila gene encoding histidine decarboxylase (HDC), the enzyme which converts histidine to histamine. Drosophila predicts from a single copy predicted to have a 60% identity to homologs in mammals. The Drosophila cDNA hybridized to an 0.4 region of the polytene chromosomes, to which the hdc gene was mapped. Using an antibody recognizing the HDC protein, antibodies to this gene that this gene encodes. The hdc gene is expressed in the insect eye. Expression is detected in the eye and in adults. Northern blot and in situ hybridizations demonstrate that the Drosophila cDNA detects 3 transcripts, expressed primarily in photoreceptors and small regions of the central nervous system. These transcripts are severely reduced in hdc mutant. The above results show that the Drosophila cDNA homolog corresponds to the hdc gene, and that mutations in this gene disrupt paired amplification. Supported by EY06214, EY03604, and EY00033.

**466.8** EXPRESSION OF TTDHVFLF Ramide, A DROSOPHILA NEURAL PEPTIDE. R. Nichols*, M. Tibbetts, and J. McCormick. Biological Chemistry and Biology Departments, University of Michigan, Ann Arbor, MI 48109.

We have generated affinity-purified antiserum to a unique portion of TTDHVFLF Ramide, a novel neural peptide from Drosophila. TTDHVFLF Ramide immunoreactivity is expressed in Drosophila brain. Two median neurosecretory cells bilaterally symmetrical to the median send projections to the brain lobes and projections down the ventral ganglion. Two anterior neurosecretory cells send projections along the midline to the brain lobes. The median neurosecretory cells arise earlier in development than the more anterior cells. Double-labeling studies indicate that the neurosecretory cells expressing TTDHVFLF Ramide are distinct from those expressing DSK peptides. TTDHVFLF Ramide DNA has been amplified from adult Drosophila RNA.
466.9
STRUCTURAL BASIS FOR PROCESSING SITE USE AND MISUSE IN AN INSECT PROHORMONE. R.C. Batur, T.J. Hone1, A. Linacre, J.D. Campbell, A. Drnik, and M. O'Shea*. Centre for Neuroscience, Univ. of Sussex, Brighton BNI 9QJ, UK.
Neuropeptidase 25, a specific protease for locust adipokinetic hormone (proAKH), has two dibasic sites, only one of which is recognised during processing in vivo.
Using computer-aided structure prediction, circular dichroism (CD) spectroscopy and 1H-2D NMR, we are studying the solution structure of a complete synthetic proAKH. CD and NMR indicate that the unused processing site is located within a region of α-helix, whereas the used processing site is not. The used site appears to be associated with a 7 residue Ω-loop in which Lys and Arg define its C-terminal neck. The importance of Lys and Arg and their positions have been studied experimentally by replacing them with their analogues thialysine and caravamine. Replacement of Lys (within the loop) but not Arg (adjacent) prevents correct cleavage C-terminal to the dibasic site. Thus, although normal cleavage is C-terminal to Arg, it does not depend on the presence of Arg, but on higher order structural features N-terminal to this site. To study how these features may interact with the enzyme, we are now cloning the cDNA of the proAKH processing endopeptidase.
1 Centre for Molecular Sci., Oxford Univ., Oxford OX 1 3QU, UK.

466.11
DAKH GENE STRUCTURE AND REGULATION OF PEPTIDE LEVEL. M.H. Schaffer* and B.E. Noyes, Dept. of Psychiatry, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235-9070.
DAKH, a member of the RPCH/RAKH family of neuropeptides, is found in insects including Drosophila melanogaster. The Drosophila gene encoding this peptide's precursor was identified using an oligonucleotide probe based on the known peptide sequence. This cloned DNA identifies a 250 nucleotide message on RNA blots which is consistent with the expected processing of this two exon gene and the message size of other family members. The general organization of the predicted DAKH precursor is identical to that of other family members: a signal peptide followed immediately by the DAKH peptide, a lys-arg processing sequence, and a carboxy terminal peptide. In contrast to the DAKH peptide, the carboxy peptide is larger [AE aa], and quite different in sequence from the homologous Manduca and grasshopper peptides, suggesting that the carboxy peptides serve some role other than binding to a receptor. The DAKH gene has been localized on salivary gland polytene chromosomes to the region E610-E641,2.
Others have identified fly strains which carry duplications or deletions of this area. Adult flies from these strains, carrying one or three copies of the DAKH gene rather than the normal two, have near wild type levels of the peptide suggesting that DAKH levels are tightly regulated.

466.13
PROCTOLIN MODULATION OF INSECT MUSCLE EXCITABILITY IS MEDIATED BY PROTEIN KINASE C. L.D. Acevedo* and M.E. Adams. Entomology Dept. Univ. of California, Riverside, CA 92521.
Proctolin is one of the transmitters at a dual-transmitter motor neuron innervating the longitudinal ventrolateral muscles (6A and 7A) of the larval housefly, Musca domestica. 6% of preparations display a muscle action potential after nerve shock or current injection into the muscle. The remaining 94% of preparations are inexcitable. In the presence of proctolin, however, these muscles display action potentials and also a change in input resistance (see also Mbungu and Adams, this meeting).
We examined the signal transduction mechanisms underlying this change in muscle excitability. Both the endogenous and the proctolin-induced action potentials were blocked by 4-bromophenacyl bromide, a broad spectrum phosphodiesterase inhibitor, indicating that hydrolysis by membrane phospholipids is involved. The phospholipase C, which mimics diacylglycerol in its activation of protein kinase C, evoked an action potential similar to the endogenous and proctolin-evoked potentials. PMCA, however, did not induce a consistent change in the muscle input resistance. This response was reversible and was not reproduced by PMA. Intracellular application of inositol trisphosphate, generated during hydrolysis of certain membrane phospholipids, did not evoke muscle action potentials. Two kinase inhibitors, H-7 and FK506, blocked both proctolin-induced and endogenous action potentials. However, membrane permeable analogs of cAMP and cGMP did not evoke action potentials. These results confirm PLC as a signal transduction pathway for the proctolin-mediated change in muscle excitability.
467.1
 des Régulations Physiologiques, Strasbourg, France.

Differentiation of cerebellar granule neurons was assessed by recording voltage-dependent ionic currents using the whole-cell patch clamp method on cells maintained in medium consisting of DMEM, 10% horse serum and 10-4 M insulin. Single pre-

cursor neurons or aggregates were removed directly from the external germinal layer (EGL). During the first 3 to 10 hours in culture (day 0), only outward currents were present; small, rapidly activating and inactivating Ia and a large, slowly inactivat-ing sustained current that partly obscured Ia, and was very sensitive to TEA. By day 5, the aggregates had spread out on the poly-

ornithine substrate; Ia5 and Ia6 had appeared in granule neurons; Ia1 increased in amplitude to dominate the sustained K current; and a second sustained outward current had appeared that was insensitive to TEA. These experiments were undertaken to provide a baseline for assessing factors that influence the differentiation and survival of granule neurons.

467.3

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The presence of EGF-responsive progenitor cells in the embryonic through to adult murine CNS (Reynolds and Weiss, Science 252:1707, 1992) prompted us to examine whether a similar cell exists in the human CNS. Embryonic human CNS tissue (cortex, striatum and cerebellum) was mechanically dissociated and plated onto untreated tissue culture flasks in serum-free culture medium containing 20 ng/ml of EGF. Dividing cells were observed within 7 days in vitro, and over the following 21 days the dividing cells formed spheres that detached from the substrate. When floating spheres were dissociated and replated at low density as single cells, proliferation was re-initiated and over a 2-4 week period new spheres were formed. When spheres were plated onto poly-1-ornithine-coated glass coverslips, cells migrated from the central core, adopting the morphology of neurons and astrocytes. The presence of neurons was confirmed with antisera directed against human neuron-specific enolase. The similarity of these findings to those we reported for the murine CNS suggest that EGF-responsive stem cells are present in the human CNS.

467.4

We have isolated a stem cell from the embryonic to adult mouse striatum (Reynolds and Weiss, Science 255:1707, 1992) which proliferates in vitro in response to EGF, forming colonies of undifferentiated cells (neuropheres) which detach from the substrate and float in suspension. Neuropheres can be mechanically dissociated into single cells and a large percentage will proliferate forming new neuropheres. The same neuropheres can be perpetuated weekly, resulting in a logarithmic growth in the number of undifferentiated cells. EGF-generated neuropheres were differentiated by mechanical dissociation and plating at high density, in the absence of EGF. After 7-14 days in vitro the three major cell types of the CNS were observed: Astrocytes, immunoreactive for GFAP, had a stellate morphology. Neurons were identified with antibodies recognizing MAP-2, neuron-specific enolase or neurofilament (168 kDa). Oligodendrocytes were immunoreactive for the cell surface antigens O4 and GC. All three cell types were present in differentiated EGF-generated progenitor cells that had been passaged for eight months (42 passages). A growth-factor-dependent CNS stem cell line may provide a continuous source of cells for intracerebral grafting.

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467.5
EGF-GENERATED MOUSE STRIATAL NEUROSHEROPHES EXPRESS THE 4&delta;3 NEUROTROPHIN RECEPTOR. J. Williams*, A. Vescey*, B.A. Reynolds* and J.P. Hammond*, E.E. Berlin* and S. Weiss*, Neuroscience Research Group, University of Calgary, Calgary, AB, Canada T2N 4N1 and 3Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT 06492.

We have identified an EGF-responsive stem cell in the embryonic and adult mouse striatum (Reynolds and Weiss, Science 252:1707, 1992), which in response to EGF will reproduce itself and generate cells that differentiate into neurons, astrocytes and oligodendrocytes. In preliminary studies, we found that when plated at low density (2500 cells/cm2), addition of EGF up to 7 days in vitro (DIV) could initiate proliferation of the stem cell, but not if applied after 7 DIV. In the present study, we sought to determine if bFGF could prolong the survival of the EGF-responsive stem cell. Striatal cells (E14, 2500 cells/cm2) were plated in the absence or presence of 20 ng/ml of bFGF. After 11 DIV, cultures were washed and media containing 20 ng/ml of EGF was added. After 4-5 DIV, in cultures that were primed with bFGF, cells examined contained clusters of proliferating cells that developed into colonies with the morphologic and antigenic properties of the EGF-generated cells we have previously described. Cultures that had not been primed with bFGF showed no EGF-responsive proliferation. These findings suggest that the EGF-responsive striatal stem cell may possess bFGF receptors that regulate its long term survival.

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467.7 GAP-43 IS DEVELOPMENTALLY REGULATED IN GLIAL CELLS DERIVED FROM EGLG-RESPONSIVE CNS STEM CELLS.
J.P. Hammond1, B.A. Reynolds2, E.E. Barge1, and S. Weiss2.
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GAP-43 is a nervous system-specific membrane phosphoprotein which is down-regulated during development. Originally, GAP-43 was thought to be neuron-specific, however, recent reports indicate that this protein may be at least transiently expressed during development in some astrocytes, oligodendrocytes and in Schwann cells. At present, the role of GAP-43 in macrogia is not known. We have begun to investigate the transient expression of GAP-43 and GFAP in glial cells generated from the EGLG-responsive stem cells derived from the embryonic and adult subventricular zone (Stromberg and Weiss, Science 255 p. 1707 1992). Astrocyte differentiation was induced by plating the stem cells in a medium containing 1% FBS with no EGF. The cells were then cultured for 14 days and stained for GFAP and using dual-label immunofluorescence. Initially, GAP-43 and GFAP expression is observed in a large number of cells. By one week however, the large majority of GFAP-expressing astrocytes no longer express GAP-43. The EGF-responsive stem cells represent a useful model system for the study of the role of GAP-43 in glial and neuronal development (supported by the MRC Canada).

467.9 CNS GLIAL CELLS SUPPORT SURVIVAL, DIVISION AND DIFFERENTIATION OF OLFACTORY NEURON PROGENITOR CELLS IN VITRO. S.E. Diksic and E. Cell and Development, Cincinnati, OH 45267.
In the adult mammal, new olfactory receptor neurons (ORNs) are produced by neurons in the olfactory neuroepithelium. To determine whether the progenitor cells could divide and differentiate in culture, we have disaggregated nasal mucosal cells from Newborn rats and cultured them on either a polylysine coating or a monolayer of neuronal rat astrocytes. On polylysine, both satellite and immature ORNs died within 5-6 days. On the monolayer of CNS astrocytes, mature ORNs (OMP+) again died rapidly (by 5-7 days), while immature ORNs (OMP-), positive for neuron-specific tubulin, began to appear and form large aggregates of neurons. OMP+ neurons reappeared 10 days after plating and were very prominent in 15 day cultures. Three different CNS stella stromas were equally effective in promoting survival and differentiation. Pulse labeling with tritiated thymidine was done over 15 days in culture. Uptake of isotope in neurons was detected by combined autoradiography and immunocytochemistry. Neuronal progenitor cells both divided and differentiated in culture. Both OMP+ and NSE+ neurons were generated by cell division that took place during restricted times after plating. Aspects of the data suggest that OMP expression is an innate property of ORNs and that expression may rely more on survival than target-specific regulatory factors. The neuronal progenitor cell appears to be negative for NSE. Astrocytes (but not conditioned medium) allow survival, division and subsequent differentiation. Support: Amer Paralysis Assoc PFI-8801-1 + NIM HCO30417.

467.11 BETAG-2 INTEGRINS ARE CONSTITUTIVELY EXPRESSED ON MICROGLIA IN THE NORMAL HUMAN FETAL BRAIN. L.A. Matthias*, E. Danks, W.D. Lyman, W. Rushing, D.W. Dickson, Deps. of Pathology and Ob-Gyn, Albert Einstein College of Medicine, Bronx, NY 10461.
To further characterize microglia in the normal human fetal brain, sections from 16 to 23 wk abortuses of HIV seronegative mothers were examined immunocytochemically with antibodies to Beta-2 integrins. Integrins are part of a group of cell adhesive molecules that are involved in cell-cell and cell-matrix interactions. As transmembrane glycoproteins, integrins link the intracellular cytoskeleton to extracellular matrix proteins. Beta-2 integrins are heterodimers that are characterized by a Mr 95,000 Beta-2 subunit (CD18) and alpha subunits of either Mr 180,000 (CD11a), 170,000 (MAC-1), 150,000 (IL-2) or 142,000 (CD11b). Antibodies to these alpha chains included LFA-1 (CD11a), B1-1/9F6 (CD11b) and Leu-M5 (CD11c). Preliminary data suggests that these markers are constitutively expressed on microglia in normal fetal human brain at 16 wk. These engravis were expressed on both ameboid and more ramified forms of microglia in the normal fetal brain and in the subependymal grey and white matter. Microglia were also found to constitutively express leucocyte common antigen, in addition to HLA-DR (L3T4, HLA-DR), a major histocompatibility complex class II antigen. These gamma delta T cells were not detected (See Neuroimmunol 17:734:1991). The role of Beta-2 integrins, as expressed on fetal microglia, in normal gliogenesis and brain development is not clear. On leukocytes, beta-2 integrins are involved in mediation of phagocytosis, adhesion and migration in the immune system. The presence of adhesion molecule receptors on microglia suggest that these cells, in the in-cell-cell and cell migration and differentiation during brain development. Since microglia are productively engaged in CNS, these receptors may also be involved in the mediation of CNS pathogenic.

The development of mammalian telencephalon involves a sequence of multiplications of stem cells, differentiation into neurons and glia, migration to target sites and synaptogenesis. Each phase is likely to involve the expression of characteristic sets of genes that specify specific gene expression and temporally-restricted manner. In order to study transcription factors orchestrating such processes, we created an in vitro model simulating aspects of early telencephalon development. Major components of the system are the: neuropeithelium of E13 rat telencephalon, grown in dissociated cell culture in serum-free medium. At the density of 80,000 cells/cm2, the addition of growth factors (bFGF and NGF) increased the number of cells (a marker for multipotential stem cells) and the proliferation rate at (5 days in vitro, bromodeoxyuridine incorporation during 3 hours labelling was 9.1±2.2 and 14.2±1.7 cells per 1000 cells, respectively). Cells containing GABA- and glutamate-like immunoreactivity started differentiating at 2 days in vitro and their number and complexity of arborization increased with time. Growth factors increased the differentiation of cells containing glutamate-like immunoreactivity at 2 and 4 DIV, while the effect on GABA-containing neurons was modest. GFRα1 receptor was strongly downregulated until day 6 but differentiated from nestin-positive stem cells afterwards. The sequence and timing of these developmental events closely matches the development of telencephalon in situ.

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467.1


A 110 kDa lamin binding protein has been shown to be expressed by enteric and other peripheral neurons. This protein is not expressed by premigratory or early migratory neural crest-derived precursor cells. Immunocytochemical observations have suggested that the 110 kDa protein might be acquired by crest-derived cells shortly after these cells colonize the developing gut of avians or mice. Experiments were carried out to test the hypothesis that the 110 kDa lamin binding protein is selectively expressed in crest-derived precursors of enticular neurons and glia. The bowel of fetal mice (E16) was dissociated and the resulting single-cell suspension was exposed sequentially to rabbit antibodies to the 110 kDa protein (α-110) and magnetic beads coated with goat-anti-rabbit secondary antibodies; α-110-immunoreactive cells were finally isolated with a magnet. α-110-selected and unselected (residual) cells were seeded in dishes coated with collagen and laminin at a density of 27 X 10^3 cells/dish and cultured for 8 days. Neurons were identified immunocytochemically with antibodies to neuronfilament proteins or neuron specific enolase, while glia were identified with antibodies to the S-100 protein. Neurons and glia were also found to be immunostained by α-110. Significantly more neurons and glia developed in cultures of α-110-immunoselected cells than in cultures of unselected cells. The proportion of cells in the cultures of selected cells that developed as neurons was about 3-fold that found in cultures of unselected cells. Similarly, the number of S-100 immunoreactive cells in cultures of selected cells was about 4-fold that observed in the cultures of unselected cells. Immunoselection of cells from E16 fetal mice thus supports the hypothesis that neurons or glia preferentially develop in cultures of cells selected with the α-110. It has been suggested that the 110 kDa lamin binding protein is a receptor that mediates the response of enteric neural and glial precursors to laminin. The current observations support this idea. Supported by NIH grants NS52766, HD17736, HD30670, HD21033 and NS15547.

468.2


The enteric nervous system (ENS) is formed by cells that migrate to the bowel from the vagal and somitic levels of the neural crest. Since the developmental potential of crest-derived cells that have entered the gut is less than that of premigratory or early migratory crest cells, the properties of at least some crest-derived cells change as they migrate to the gut. In order to study the characteristics of crest-derived cells, it is necessary to isolate them from within the bowel. Gut from chick or quail embryos (E4-7) or fetal rats (E11-15) was dissociated with collagenase to yield a suspension of single cells, which were then subjected to immunoselection. The suspension was mixed with a murine monoclonal antibody (NC-1) that reacts with a surface epitope that is found in the gut, only on crest-derived cells. NC-1 coated cells were then isolated with magnetic beads coated with goat anti-mouse antibodies, and selected with a magnet. NC-1* cells were cultured for up to 8 days, as were the cells remaining after the NC-1 population had been removed. Immunoselection was found to greatly enrich the population in NC-1* cells. Expression of neurofilament proteins and neurite extension were preferentially seen in cultures of immunoselected cells. Neurite outgrowth was not inhibited by laminin. Some neurons contained 3-aminobutyric acid (GABA) and serotonin (5HT); however, tyrosine hydroxylase (TH) and dopamine-B hydroxylase (DBH) are not expressed by avian enteric neurons, and are expressed only transiently in developing rat bowel were present in immunoselected cells. A glial marker, glial fibrillary acidic protein (GFAP), was apparent in a subset of immunoselected cells. Non-selected cells proliferated rapidly and relatively few expressed a neuronal or glial phenotype. These experiments demonstrate that neurogenic crest-derived cells can be isolated from within the enteric mesenchyme of both developing rats and birds. At least some of the immunoselected cells appear to be capable of expressing phenotypes appropriate for the ENS. The appearance of TH in cells immunoselected from avian bowel supports the hypothesis that the enteric microenvironment acts to suppress cholinergic expression in situ. Supported by NIH grants HD20470, NS15547, NS26766, HD17736, and a fellowship from Lily Res. Lab.
468.5
SPECIFICATION OF THE ROSTROCAUDAL AXIS OF THE PREMIGRATORY AVIAN NEURAL CREST. Gabrielle G. Leblanc*.
Dept. of Biological Structure and Function, School of Dentistry, Oregon Health Sciences University, Portland, OR 97239.

Different rostrocaudal populations of premigratory neural crest cells differ in their developmental potentials, both in vitro and in vivo. In vitro, cranial neural crest gives rise to large amounts of cartilage and bone, whereas trunk neural crest cells lack chondrogenic potential. In vivo, cranial neural crest produces large numbers of fibroblasts (FN- and procollagen I) (Col I)-immunoreactive cells, whereas trunk neural crest produces relatively few cells. These in vitro differences in protein expression by cranial and trunk neural crest cells can be used to explore the mechanisms that determine the rostrocaudal axis of the premigratory neural crest.

The rostrocaudal axis of the premigratory neural crest may be specified by factors acting on the underlying somite. To test this possibility, I examined whether crest with anterior mesoderm can induce trunk neural crest cells to express cranial-specific traits in vitro. Trunk neural crest cells were cocultured together with explants of early (stage 3) Hensen's node, which contains prospective anterior mesodermal cells. After five days of coculture, both FN and Col I immunoreactivities were seen in the neural crest cells surrounding the Hensen's node explant. The apparent FN- and Col I-stimulating activity of early Hensen's node is mimicked by transforming growth factor (TGF)-B.

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468.6
BASIC FIBROBLAST GROWTH FACTOR (bFGF) INFLUENCES NEURAL CREST CELL FATE VIA AN INTRACRINE MECHANISM. G. Cimera*, L. Sherman, K.M. Stocker and R.S. Morrison.

In previous work, we found that neural crest (NC)-derived Schwann cell progenitors of early avian embryos underwent a transformation in vivo or in culture into another NC lineages - melanocytes. In the following treatment with either the phosphor elster TPA or basic fibroblast growth factor (bFGF). These and other data suggest that bFGF might be involved in the commitment of bipotent intermediate cells in the NC lineage, either the melanocyte or Schwann cell fate.

In these studies, we show that TPA induces bFGF expression in the melanocyte/Schwann cell progenitors, but that bFGF need not be released in order to induce influence cell fate. Treatment of cultures of early embryonic peripheral nerves with TPA, for example, induced expression of bFGF mRNA and protein and caused melanogenesis in cells which would normally have given rise to Schwann cells. Addition of two different bFGF antisense oligonucleotides blocked these effects of TPA, but that sense oligonucleotides or scrambled oligonucleotides (i.e., with the same base content but with a different sequence) did not, suggesting that bFGF expression is part of signalling pathway by which TPA induces transformation into melanocytes. Additon to the culture medium of bFGF neutralizing antibodies or other agents known to inhibit bFGF-binding to its extracellular receptor had no effect on the TPA-induction of melanogenesis, indicating that bFGF does not need to be released in order to act. These data indicate that bFGF may act as an intracellular "intracrine" factor in the determination of NC cell fate.

468.7
INVESTIGATION OF THE ROLES OF TGFβFGF AND FGF SIGNALING IN RETINAL DEVELOPMENT THROUGH ALTERNATIONS IN RECEIVER EXPRESSION. L. Lillien* and C. Cepko. Department of Genetics, Harvard Medical School, Boston, MA 02115.

In order to begin to identify the signaling systems that regulate the development of the rat retina, we used in vitro assays to screen for extracellular signaling molecules that modulate proliferation and cell type choice. These functional assays showed that several peptide growth factors found in the developing retina, including TGFβ and FGFs, enhanced proliferation and selectively inhibited rod photoreceptor development (Lillien and Cepko, Development 1992). The effects of TGFβ and FGF on proliferation were found to be temporally restricted in vitro; cultures of younger retinal cells (<E20) were more responsive to FGF while cultures of older retinal cells (=>E30) were more responsive to TGFβ.

To determine the roles of these signaling systems in normal retinal development, we are using retrovirus vectors to express the human EGF receptor and mouse FGF receptor-1. To analyze the function of endogenous EGF and FGF receptors, we are introducing truncated forms of the receptors to block function in a dominant-negative manner.

To determine whether limiting levels of receptor expression underlie the temporal changes in responsiveness to TGFβ and FGF observed in vitro, we are introducing full length forms of the receptors. In order to label expressing these constructs, PGF R1 viral constructs also contain the gene encoding the biochemical marker alkaline phosphatase. Cells infected with the EGF receptor virus can be distinguished with an antibody that selectively labels the human form of the receptor. Choroid of infected cells will be examined.

468.8
RETNAL LINEAGE OF THE CLEAVAGE STAGE PROGENITOR IN XENOPODIS IS DEPENDENT ON POSITION. S. Huang* and S.A. Moom*.
Dept. Anatomy & Cell Biology, Univ. Virginia, Charlottesville, VA 22908.

Our previous studies (Neurobiol, Abstr., 17294, 1991) demonstrated that the retinal lineages of cleavage stage blastomeres change after the ablation of the major retinal progenitor and the changes are different for dorsal and ventral blastomeres. To determine whether the retinal lineage changes are due to the position of the remaining blastomeres two sets of experiments were carried out. First, several different sets of blastomeres were ablated at 32-cell stage embryo to determine whether the change in position of the remaining blastomeres predicts their change in retinal fate. Along the dorsal midline where a blastomere was ablated its animal neighbor took over its retinal fate and produced an amount of retinal cells typical for the ablated vegetal neighbor. When more ventral blastomeres were removed, the dorsal blastomeres did not adopt the retinal fates of their ventral/animal neighbors. These results demonstrate that the blastomeres shift fate in a dorsal/ventral direction but not in a ventral/animal direction. In order to directly test whether blastomere position determines retinal fate, reciprocal transplantations were done between a dorsal midline blastomere, the major retinal contributor, and a ventral blastomere, which normally only gives rise to ventral trunk structures. The transplanted blastomeres adopted a new fate according to their new position. The ventral blastomere became a major retinal contributor when it was placed in the dorsal animal pole, while the clone of the major retinal progenitor was restricted to the tail region of the embryo. These results were used in the position that a blastomere prior to gastrulation is the important determinant of whether retinal cells are among its descendents.

Supported by NS23158 and EY09402.
468.11
CLONAL HETEROGENEITY IN THE GERMINAL ZONE OF THE DEVELOPING RAT TELEENCEPHALON. S. E. Askin* and D. van der Kooi. Dept. Anatomy, Univ. Toronto, M5S 1A8, Toronto, CANADA.

In order to characterize the proliferation characteristics of precursor cell lines in the mammalian telencephalic germinal zone, we have previously employed a simultaneous double labeling technique which combines the visualization of individually proliferating clones in the E18-E19 developing rat telencephalon and their double labeling with 3H-thymidine. After 48 hours survival we found the cortical (but not the striatal) germinal zone to be segregated into three spatially distinct horizontal bands (A, B, and C), one of which contained clonally related populations with distinctive cell cycle times, incidences of cell death and modes (asymmetric vs. asymmetric) of proliferation (Askin & van der Kooi, soc. report). It is important to ask whether these distinct cortical germinal populations project the mature phenotype (e.g. neurons vs. glial) or spatial identity (e.g. deep vs. superficial cortical layers) of the cells they give rise to. In a first set of experiments we characterized the space distribution and proliferation characteristics of cortical precursor populations (using our double labeling method) after only 17 (instead of 48) hours survival. We found that although the overall thickness of the germinal zone was slightly smaller after 17 hours survival (E17-18), it was still segregated into horizontal clones (A, B, and C) with comparable proliferation characteristics to those seen at E19. We also found that with the longer survival of 48 hours (E17-19) compared to the shorter survival limit of 17 hours (E17-18), the clonal population in the ventricular zone proper (A) and (B) of two horizontal bands in the subventricular zone increased in thickness, whereas the second horizontal band (C) in the subventricular zone decreased substantially in thickness. Thus, clonal population C (perhaps giving rise to a subpopulation of neurons) may have become more prominent at E20. It is proposed that the different cell populations in the germinal zone remain parasitically, when neurogenesis has largely ceased but glia still are being produced. The identification of this remaining population (presumably giving rise to glia) by its proliferation characteristics may suggest an isochronism between it and one of the earlier germinal zone populations.

468.12

We reported recently that early in cortical neurogenesis (E15/E16), the ventricular zone in the rat contains separate progenitor cells for the principal neuronal cell classes, the pyramidal and nonpyramidal neurons. Here we sought to investigate whether these neuronal types originate from separate progenitor cells throughout the period of neurogenesis. For this purpose, recombinant retrovirus vectors containing the genes for E.coli β-galactosidase were injected into the telencephalic ventricles of rat embryos at E14/E21. Serially cut coronal sections of adult cortex were histochemically stained for β-galactosidase and processed for electron microscopy. Camera lucida drawings were made to map the position of labelled cells. Discrete clusters of closely spaced labelled cells were considered to be derived from the same precursor cell in the ventricular zone, i.e. to belong to the same clone. Clonally-related labelled neurons were examined with the electron microscope, and their phenotypes identified using ultrastructural criteria.

Clusters of clonally-related neurons examined from animals injected with retrovirus at various times during corticogenesis contained at all times either all pyramidal or all nonpyramidal neurons. These findings suggest that the ventricular zone contains separate progenitor cells for pyramidal and nonpyramidal neurons during the entire period of neurogenesis.

468.13
NEUROTTRANSMITTER EXPRESSION IN CLONALLYRELATED NEURONS IN THE RAT CEREBRAL CORTEX. M.C. Mione, C. Danesi, M.E. Cavanagh, P. Boardman and J. Parnavelas (SPON Brain Research Association). Department of Anatomy, University College London, London WC1E 6BT, U.K.

We examined the neurotransmitter content of clonally related neurons in the cerebral cortex of adult rats. Such neurons were marked with a retrovirus lineage tracer, containing the reporter gene for E.coli β-galactosidase, injected into the telencephalic ventricles of rat embryos at E15-E17 and subsequently histochemically. Discrete clusters of β-galactosidase positive cells, considered to be clonally related, were subjected to immunohistochemical analysis for the inhibitory neurotransmitter GABA and the excitatory amino acids Glu and Asp. This analysis was performed on Araldite embedded, 0.5 μm thick sections. Three consecutive sections through every neuron of 30 discrete clusters of neurons were processed each for one of the three neurotransmitter candidates, while thin sections were used to identify the phenotype of every cell with the electron microscope. Clusters contained between 2 and 8 neurons which were either clustered on one layer or distributed over 2 or more layers.

Nineteen clusters contained neurons positive for only one transmitter candidate (GABA, 5 Glu, 3 Asp), and one group of 3 nonpyramidal neurons was immunonegative for all 3 amino acids. However, in the clusters positive for Glu or Asp a number of neurons contained both excitatory amino acids. These findings support the hypothesis that genetic determinants play an important role in the expression of the principal neurotransmitters in neurons of the mammalian cerebral cortex.

468.14

Radial glia and differentiated myelin-forming oligodendrocytes are present in the human fetal spinal cord (HFSC) by 10 weeks of gestation (WOG). However, the neuroanatomic correlation of the development of these two cell types has not been performed. In this study, immunohistochemical and histochemical methods using antibodies to vimentin and glial fibrillary acidic protein (GFAP) were used to mark radial glia and an antibody to myelin basic protein (MBP) identified oligodendrocytes. Myelin in the vibratome sections of 37 HFSC ranging in age from 9-20 WOG. Radial glia were more numerous in the anterior and anterolateral funiculi than the dorsal funiculi; the posterolateral funiculus had very few radial processes. In addition, radial glia were almost always more numerous at the cervical level. Expression of MBP followed the same pattern. The results suggest that radial glia and oligodendrocytes follow anterior-to-posterior and superior-to-inferior developmental patterns. These gradients appear to be independent of the tracts of the developing spinal cord. Interaction between these cell types may be necessary for appropriate development of each. Further work is necessary to elucidate this interaction.

Supported by USPHS MH 47667, MH 46815 and DA 05083.

468.15
THE TIMING OF MOTONEURON COMMITMENT IN THE DEVELOPING CHICK SPINAL CORD. M.P. Main* and C. Lance. Dept. of Neurosciences, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

When 3·4 Lumbosacral (LS) spinal cord segments (+-notochord) are reversed about the rostrocaudal (R/C) axis in stage 15-16 chick embryos, motoneurons which emerge from these reversed segments alter their peripheral course to project to their originally inappropriate muscle targets. This finding suggests that there are differences along the R/C spinal cord axis at neural tube stages (stages 15-16) and that the motoneurons within LS segments are commited to a particular peripheral target fate when their axons grow out of the cord (stages 18+). How and when are motoneurons committed to specific target areas? We have begun to address this question by carrying out R/C cord reversals at earlier stages.

At stages 13-14, presumptive LS cord segments I-3 were separated from the notochord and reversed about the R/C axis. At stages 35-37, retrograde HRP labelling was used to define the R/C location of motoneurons of the vagus, thoracic and lumbar somites. Motoneurons innervating these muscles are normally found in discrete positions within LS 1-3. In 70% of stage 13 cases (n=14) and 55% of stage 14 cases (n=9), motoneurons innervating these muscles were located in discrete positions in accord with their new R/C location. In 30% of stage 13 cases and 32% of stage 14 cases, motoneurons were dispersed throughout the reversed segments. In only one case (at stage 14) did motoneurons project in accord with their origin prior to the reversal. These findings suggest that R/C differences within the spinal cord that lead to motor neuron commitment are not present at stages 13 and 14. (Supported by NIH HD 256769).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
469.1 SYNAPTIC INPUT TO TRANSIENT SUBCORTICAL SYNAPTIC ZONE (TSS) NEURONS IN INFRAGRANULAR CORTEX: PROVISION OF DIRECT SYNAPTIC INPUT TO UNDERLYING TSS.
A.P. Sterling and P.R. Lomstein*. Laboratory of Cellular and Developmental Neurobiology, Dundee University Institute of Medical Biology, DD1 4HN, Scotland.

In the neonatal rat cerebral cortex antibodies raised against synapsin I and synaptophysin reveal a TSS beneath the developing cortex. This synaptic neurolill appears as the animal matures. We have examined the source of synaptic input to the TSS by immunocytochemically injecting the anterograd e tract tracer physalin into the infragranular somatosensory cortex. PHA-I, was visualised using immunohistochemistry and sections were prepared for light and electron microscopy (EM). Terminal arborization of the intracortical neurone was evident in the lateral geniculate nucleus. Fibres traversing the TSS were seen to be varicose and when examined under the EM many varicosities were found to establish asymmetric synapses:
Age: Target structures Synaptotagmin synapses
< 10 days 85% 15%
1 month 60% 35%
Adult 100%

These results demonstrate that neurones located in the infragranular layers of the developing cortex provide direct synaptic input to subcortical TSS neurones. The developmental transition of post-synaptic targets from spines to shafts suggests that cortico-TSS axo-spinous synapses are indeed a population of transient synapses.
Supported by The Wellcome Trust, Royal Society and University of Dundee.

469.2 NEOCORTICAL SYNAPTOSTEMOSIS: LOW DENSITY PRIMARY CULTURES AND USE OF HEK-293 VECTORS FOR GENE TRANSFER INTO POSTMITOTIC NEURONS AND GLIAL CELLS. P.R. Lomstein, T. Hodges, N.D. Stone*, C.M. Preston, and M.G. Castro*, Dept. of Anatomy- Physiology, University of Dundee, DD1 4HN, MRC Institute of Neurology, University of Glasgow, G11 5JR, and Dept. of Molecular Life Science, Dundee Institute of Technology, Dundee DD1 4HG, Scotland.

Neocortical synaptic organization is very specific in terms of the post-synaptic targets of individual neurones, and the synaptic input onto individual cells. Although cortical neurones have 5 different membrane domains on which they could potentially receive synaptic input, neurones tend to prefer fewer classes of synaptic input. Also, excitatory cell bodies only receive inhibitory synaptic input, while inhibitory somata receive both excitatory and inhibitory input. We now wish to examine the cellular and molecular basis of the selection of synaptic inputs by using a very low density primary culture system of neocortical neurones, based on the work of Banker, Konik, and Ramanek, we determined that neurones: (a) survive for long term in low density; (b) expand; and, of absence of contact; (b) develop proper morphological polarity (e.g. dendrites and axon); (c) after 3 weeks in vitro MAP-2 is found only in dendrites, while is localized to all cellular processes; (d) immunolocalization of synergistic synapses co-localized with confocal microscopy suggests that the pattern of synergistic in vitro is comparable to the in vivo one; (e) HEK-293 vectors can be used to transfer foreign genes into both postmitotic neurones and glial cells in primary culture. Thus, in spite of its intrinsic complexity in vivo, the cellular and molecular basis of neocortical synaptic formation can now be examined using a simple in vitro model.
Supported by The Wellcome Trust, MRC, SERC, Royal Society, University of Dundee, Smith Kline 1982 Foundation and Dundee Institute of Technology.

469.3 DEVELOPMENT OF SYNAPTIC CONTACTS BETWEEN NEURONS OF BASAL FOREBRAIN AND CEREBRAL CORTEX IN ORGANOTYPIC TISSUE SLICE CULTURE. P.G. Distler & R.J. Robertson*. Department of Anatomy and Neurobiology, University of California, Irvine, 92617

We have previously shown that AChE-positive neurones of rat basal forebrain project into cultured cerebral cortex slices and, after entering the tissue, branch within the different cortical layers (Distler and Robertson, 1992). We are now investigating whether these projections also form synaptic contacts with neurones in the cortical target region.
Slices of neonatal basal forebrain tissue were co-cultured with 4 day old cerebral cortex tissue for up to 4 weeks. Various developmental stages of the basal forebrain-cerebral cortex innervation were then investigated on the fine structural level. For identification of axon terminals, two different labeling techniques were employed: (a) placement of the lipophilic dye Dil on fixed basal forebrain tissue and subsequent DAB-phoatoenzyme of stained fibres with cortical tissue, and (b) the cholinergic marker, a label which is known to be a reliable marker for cholinergic basal forebrain neurones.
The electron microscopic investigation revealed consistent results for both staining methods. First, basal forebrain neurones form terminal synaptic contacts on dendritic shafts in the superficial cortical layers. Second, the observed synapses are of the symmetric type. Furthermore, both methods reveal the presence of clear spherical vesicles in the presynaptic profile. Within superficial cortical areas, stained synaptic contacts were evident as early as one week after beginning culturing.
In conclusion, the data reveal parallel to the cholinergic innervation in vivo, and provide further evidence about the significance of this model system for the study of the development of the cerebral cortex.
(Supported by NIH grant NS 25674, Alzheimer Foundation grant 90-082 and Deutsche Forschungsgemeinschaft grant Di 4451-2).

469.4 INTERRUPTION OF AXONAL GROWTH BY TARGET NEURONS IS ENHANCED BY NMBA. D.H. Baird*, A.D. MacDermot*, E. Trenkner*, and C.A. Mason*. "Howard Hughes Medical Institute, Rockefeller Univ., NY State Institute for Basic Research in Developmental Disabilities; Dept. of Pathology and Physiology and Cell and Biophysics, College of Physicians and Surgeons of Columbia University, New York, N.Y. 10032.

 Cultured cerebellar granule neurones interrupt the growth of their mossy fibre afferents originating from Purkinje neurones. Using the anterograde label di-benzamidine (DBA) at 92 J. Neurosci. 12:619-634). This "stop-growing signal" is afferent and target cell specific (Baird et al. J. Neurosci. 1989; 9: 3023-3031), and affects neurones in the target volume. Action potentials and neurotransmitters play a role in the stop-growing signal as TTX, TTX + high magnesium, or kynurenic acid, an antagonist of glutamate receptors, all interfere with the stop-growing signal. To determine which types of glutamate receptors might be involved in the stop-signal, explants of pontine nuclei were co-cultured on granule neurones for two days in the presence of glutamate agonists and antagonists specific for NMDA and non-NMDA receptors. The NMDA-specific antagonist D-AP5 interferes with the stop-signal, resulting in a large increase in the number of pontine neurones extending over granule neurones compared to controls in medium. In the presence of 200uM NMDA the number of long neurones was reduced to less than 40% of controls. Pontine explants cultured without granule neurones but with NMDA showed a small increase in the number of long neurones produced. In contrast, explants co-cultured with granule neurones in 50uM AMPA (a non-NMDA agonist) showed little reduction in the number of long neurones produced. AMPA had no effect on the number of pontine neurones in the absence of granule cells.
These results suggest a role for NMDA receptors in regulating the growth of mossy fibres on target granule neurones and potentially in contributing to target cell specificity during the innervation of the cerebellum.


Granule neurones in vitro present a stop-growing signal for their appropriate afferents, the mossy fibres. This signal is afferent-specific since neither retnal nor olfactory neurones receive the stop signal (Baird et al. J. Neurosci. 1992 12:619-634; J. Neurol., in press).

With the aid of the anterograde tracer DBA (Baird et al. 1991 Soc. Neurosci. Abstr. 17:38), we examined the capacity of this cell type to regulate afferent growth. To study the growth of one afferent to Purkinje cells, the parallel fibres afferents to granule neurones, we co-cultured aggregates of purified granule cells with purified Purkinje cells. Purkinje cells stimulate and maintain the growth of granule afferents, in line with previous reports of parallel fibres across the dendritic fields of multiple adjacent Purkinje cells. In turn, granule neurones and their axons maintain and promote the differentiation of Purkinje neurones. Granule cells, and granular neurones have different modes of regulating afferent growth, in some cases stimulating growth.

Second, we observed how an inappropriate afferent, the pontine mossy fibres, can affect the growth of Purkinje cells. Mossy fibre growth is abundant on a number of substrates and is not affected by co-culture with Purkinje cells. However, when grown with granule neurones, mossy fibre growth is increased three fold compared to growth on granule cells alone. While it is not known if Purkinje cells stimulate mossy fibre growth directly, or disrupt the stop-signal for mossy fibre growth by interacting with granule neurones, these results suggest that Purkinje cells do not send a stop-signal to mossy fibres and that this is afferent-specific.

The above paradigms will allow the analysis of mechanisms involved in the specificity of axon growth regulation, its maintenance by target cells and the establishment of specific synaptic connections.

469.6 SPATIAL DISTRIBUTION OF EXCITATORY AND INHIBITORY SYNAPSES ON A PURKINJE CELL IN A RAT CEREBELLAR CULTURE. T. Hirano*, K. Kajos, Department of Physiology, Faculty of Medicine, Kyoto University, Yoshida Konoe-cho, Sakyo-ku, Kyoto 606, Japan.

Spatial distribution of synapses on Purkinje cells formed in a dissociated cell culture of rat cerebellar cortex was studied by intracellular fluorescent stainings of pre- and postsynaptic neurones and by immunocytochemical staining of presynaptic terminals. Simultaneous whole-cell recordings were performed on both a presynaptic small neurone and on a Purkinje cell, and the property of synaptic transmission was determined. Instead excitatory input to Purkinje cell was suppressed by CNQX, and outward inhibitory synaptic currents were suppressed by bicuculline, respectively. A presynaptic neurone was stained with intracellularly injected Lucifer yellow and presynaptic terminals of inhibitory interneurons such as basket or stellate cells were found both along dendrites and on the soma. After the electrophysiological recordings, neurones were filled with biocytin and immunocytochemistry using anti-synaptophysin monoclonal antibody in order to visualize overall distribution of synaptic terminals on a Purkinje cell. The staining confirmed that the varicosities are presynaptic terminals and a Purkinje cell receives synaptic inputs both on a soma and on dendrites. Thus, differential spatial distribution of excitatory and inhibitory synapses on a Purkinje cell in a simple cell culture system was demonstrated. Synapses were also observed with a scanning electron microscope.

2) Channels and postsynaptic receptors. AM (8 uM). Focal application of either 50 mM K+ or 100 uM glutamate inhibited the occurrence of synchronous oscillation after 8 days in culture, others (Ito, M., 9510, 1989) to the cortical culture. Continuous presence of EGCase together with its activator protein from ministry of Education, Science and Culture of Japan.

3) We have carried out quantitative analysis of synapse formation between cultured CNS neurons using multi-site Ca2+-fluorometry and electron microscopy (Kuroda et al. Neurosci. Lett. 135:255, 1992). Synaptophysin Immunostaining using laser confocal microscopy demonstrated that neurons were connected by many synapses which matured during the culture (Ichikawa et al., Neurosci. Res. 12:452, 1991). To investigate whether these synapses are formed between specific pairs of neurons in culture or not, we attempted to identify different types of the CNS neurons. We developed a library of monoclonal antibodies which bind to living PC12 cells differentiated by NGF treatment. At least 14 monoclonal antibodies in the library bound almost exclusively to living neurons from rat hippocampus in culture. We also screened a series of lectins to the living neurons. Vicia villosa agglutinins (VVA) binding appeared to be significant to multipolar neuron in the hippocampus after 2 weeks in culture. These neurons appear to be inhibitory, which identified in fixed hippocampal tissue (Drake et al. Brain Res. 554:176, 1991).


The possible involvement of proteolysis in the process of activity-dependent synapse elimination was studied in an in vitro model of the neuromuscular junction. Neurons of the superior cervical ganglia (SCG) and muscle cells were isolated from newborn mice and cultured in a multicompartiment system, the Campenot chamber. The SCG neurons were kept in the two side compartments; muscle cell was in the center. Cholinergic synapses developed between neurons and muscle cells after 2-3 weeks. Functional synapses were monitored by the contraction of muscle cells in response to stimulating theafferent neuron. The elimination of these synapses was activity-dependent. After 1-2 days of extracellular stimulation at an average of 576 spikes, the synaptic connections were eliminated (n=275). When 50 uM leupeptin, a protease inhibitor, was added into the central compartment during the whole period of stimulation, only 35% of the synapses were lost (n=193). The difference between the effect of stimulation in the presence and absence of leupeptin was statistically significant (p<0.001). Synapse elimination in unstimulated controls was 8% (n=117). In the presence of 1 uM tetrodotoxin (TTX), stimulation did not cause synapse elimination (n=107).

In conclusion, the partial block of synapse elimination by this protease inhibitor suggests that proteolysis may play a role in the mechanism of activity-dependent synapse elimination.

469.10

SOMATOTOPIC ORGANIZATION OF OVERLAPPING MOTOR UNITS WITHIN A LARVAL FROG JAWS: E.F. Omerza and K.E. Alley, Ohio State University, Columbus, Ohio 43210.

Individual myofibers within the larval jaw muscles of Rana pipiens are polyinnervated. This is due to the presence of multiple neuromuscular junctions (NMJs) as well as multiple axons within individual junctions. The purpose of this study was to determine whether this polyinnervation arises from more than one neuron and, if so, to investigate their central and peripheral organization. To determine the neuronal distribution at muscle, myofiber and NMJ levels, we employed a triple labeling technique utilizing standard ACHE histochemistry and the anterograde fluorescent tracers Dil and DiA. This procedure allowed us to distinguish the distribution of two separate groups of axons to their respective NMJs. A second study used retrograde axonal transport of Fast Blue and Dil from injection sites at opposite ends of the muscle. This provided the location of their territories serving different regions of the same muscle. Results of the triple label study indicate that individual NMJs and myofibers may receive innervation from axons within different portions of the nerve root, and further, suggests a regional distribution for motor units to their respective NMJs. We have also demonstrated that neurons projecting to the rostral and caudal regions of the muscle are distinct. Our observations indicate that several neurons can project to a single muscle fiber or NMJ and that the resultant overlapping motor distribution is reflected in a somatotopic organization of motoneurons.
4.7.1

EFFECT OF IN UTERO ETHANOL EXPOSURE ON THE MORPHOLOGICAL DEVELOPMENT OF THE OCULOMOTOR NUCLEUS IN THE RAT.


This study was designed to determine if exposures to ethanol can alter the development of brainstem nuclei controlling the extraocular eye muscles. Pregnant rats were fed through gestation with either an ethanol containing diet, in which 7.5% of the total caloric content was ethanol derived, or with an isocaloric diet. Male offspring were perfused on postnatal day 15 and the oculomotor nucleus was examined using plastic section light microscopy, Golgi-Cox staining, and stereologic analysis of electron micrographs. A decrease in the number of neurons as well as an increase in the number of astrocytes per unit area was found in the ethanol exposed animals, while the total number of cells per unit area remained relatively constant. There was an alcohol-induced decrease in the overall complexity of the dendritic ramifications, as well as in the total number of dendrites. The area of the neuronal soma was also decreased in the alcohol exposed animals, but there was no change in the area of the nucleus or nucleolus. These results may help explain some of the motor deficits associated with the visual system after developmental alcohol exposure.

Supported by NIAAA AA7042 and NSF EPSCoR RII-8921978.

4.7.2

TEMPORAL CONSTRAINTS ON ALCOHOL-INDUCED CORTICAL ASTROGLOSIS DURING THE NEONATAL BRAIN GROWTH SPURT IN RATS. J.T. Leng, E. Goodlett, and J.R. West, Alcohol and Brain Research Laboratory, Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

It has previously been reported that alcohol exposure during the brain growth spurt (postnatal days P1-49) in a pattern yielding cycle blood alcohol concentrations with high peaks and low troughs, produces a conspicuous reactive gliosis in the cerebral cortex. Radioimmunoassays have shown that the amount of GFAP is increased more than 300% above controls. Immunocytochemical studies indicated that much of the increased GFAP was associated with loci of reactive astrocytes surrounding some cortical blood vessels. These loci, scattered throughout the cortex, contained hypertrophied astrocytes with thick fibrillary processes heavily labeled by GFAP. The goal of this study was to determine if a similar pattern of alcohol exposure later in the neonatal brain growth spurt (PD 10-14) would stimulate astrogliosis comparable to exposure on days 4-8. Pups were gastrostomized either on PD 4 or PD 10 and were given either 4.5 g/kg of alcohol per day (delivered in 2 of 12 daily feedings as a 10.2% (v/v) solution in milk formula) or were given a control matched diet free of alcohol. Pups were perfused on postnatal day 9 or postnatal day 15 and 40 µm thick frozen coronal sections were processed for GFAP immunoreactivity using peroxidase-antiperoxidase immunocytochemistry. Matched sections from control and alcohol-treated pups were evaluated microscopically for reactive gliosis around cortical blood vessels. In contrast to the prominent effect seen in pups exposed on PD 4-8, alcohol exposure on PD 10-14 did not result in the intense reactive astrogliosis surrounding cortical blood vessels. These results indicate the presence of a temporal window of vulnerability to alcohol-induced reactive gliosis existed around cortical blood vessels. The critical period for this effect appears to end by postnatal day 10. Supported by grants AA 070313 and AA 05523.

NUTRITIONAL AND PREGNATAL FACTORS: ALCOHOL

4.7.4

ALTERED DEVELOPMENT OF DOPAMINERGIC NEURONS IN THE RAT SUBSTANIA NIGRA FOLLOWING PRENATAL ETHANOL EXPOSURE. A.K. Shetty, R.C. Burrows, and D.E. Phillips, Dept. of Biology, Montana State University, Bozeman, MT 59717.

The development of dopaminergic neurons of substantia nigra pars compacta (SNc) was investigated following prenatal ethanol exposure. Pregnant rats were either fed with an ethanol containing liquid diet (6.7% v/v) or were pair fed an isocaloric diet throughout gestation. Offspring were sacrificed on postnatal day 15 and the morphology of neurons was assessed by tyrosine hydroxylase (TH) immunocytochemistry and by Golgi-Cox staining. Compared to control offspring, ethanol exposed offspring had more densely packed and smaller TH positive cell bodies, fewer dendrites, and fewer TH positive fibers, reduced numbers of second, third and fourth order dendrites, fewer total dendritic segments per cell, and an altered dendritic branching pattern. The results also indicate that ethanol exposure causes retardation of the development of SNc neurons, especially of the growth and branching of dendrites. The underdevelopment of dendrites could result in altered development of neuronal circuitry which in turn could contribute to the abnormal motor functions reported after developmental alcohol exposures. Supported by NSF EPSCoR RII-8921978 and NIAAA AA7042.

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648.3

EFFECTS OF ALCOHOL EXPOSURE DURING DEVELOPMENT ON THE NEUROCHEMISTRY OF THE AMygDA.LA. Sandra J. Kelly. Department of Psychology, University of South Carolina, Columbia, SC (U.S.A. 29008).

Previous work from this laboratory has suggested that the amygdala may be affected by exposure to alcohol during the early postnatal period. This period is similar with respect to brain growth to the third trimester in humans. The alcohol exposure is given via an intracerebral rearing procedure in which rats are exposed to either 0, 0.5, or 3 g/kg/day of ethanol from postnatal days 4 to 10. Control groups consist of rats naturally reared but not exposed to alcohol and rats reared normally by dams. At 21 days of age, the rat brains were killed and their heads were immediately immersed in liquid nitrogen. The amygdala region was dissected free, sonicated in 0.2 M perchloric acid, and frozen until time of assay. At that time, the sonicated tissue was thawed and centrifuged for 15 min. The supernatant was analyzed for noradrenaline, dopamine, DOPAC, HVA, 5-HT, and 5-HIAA content.

Exposure to alcohol during development alters the amygdala in 21 day old rats. Males were in rats of both sexes and the 5-HT levels were increased in female rats. There were also a number of effects of artificial rearing on neurochemistry. (Supported by NIAAA Grant AA08380)

648.4

EXPRESSION OF LHRH mRNA IN THE C57Bl/6J MOUSE FOLLOWING ACUTE IN UTERO ETHANOL EXPOSURE. B.C. Scott*, R.T. Zoeller, P.K. Ruden, Department of Anatomy and Neurobiology, University of Missouri School of Medicine, Columbia, MO 65212.

Previously we have shown that an acute dose of ethanol on gestational day 7 (G7) alters the number of neurons immunoreactive for LHRH peptide (Scott et al., Dev. Brain Res. 66:119, 1992). The effect of an acute dose of ethanol on G10 on the expression of LHRH mRNA was examined in this study. G10 corresponds to the day of development when LHRH neurons begin to differentiate as a single neuronal population in the medial olfactory placode of the mouse (Wray et al., Proc. Natl. Acad. Sci. U.S.A. 86:3132, 1992). C57Bl/6 mice were exposed to two doses of 25% ethanol (2.9 g/kg body weight) on G10. Control pups were intubated with water. Animals were sacrificed on G18 and frozen coronal sections cut at 12 μm. In situ hybridization histochemistry was used to identify individual neurons containing LHRH mRNA. A Bioquant MEG IV image analysis system determined the area covered by grains for each neuron expressing LHRH mRNA in the diagonal band of Broca/medial preoptic area. Fetal ethanol exposure does not appear to interfere with LHRH gene expression when given during an important period of LHRH neurogenesis. (Supported by NIAAA grants AA07458, AA05893 and AA00107)

648.8


Maternal alcohol consumption (36% ethanol-derived calories during the last two weeks of gestation) produces a long-lasting alteration in stress-induced neuroendocrine and neuro-behavioral responses and in basal (non-stressed) neuroimmune responses in the offspring. In order to determine the effect of FAE on stress-induced LHRH expression in the male Sprague-Dawley FAE and control rats were exposed to swim stress (5 periods of 3-min swim in 37°C water over 30 min, with tails weighted). One hr later thyroxines were removed and their proliferative response to Concanavalin A was tested. The thymoproliferative response of swim-stressed control rats dropped by 69% compared to unstressed controls (p<0.01), while it decreased by only 40% in unstressed FAE rats. The ontogenic and temporal profiles of this FAE-induced difference in cellular response to stress are currently under investigation. (Supported by VA Medical Research Service.)

648.6

THE INFLUENCE OF CHRONIC PRENATAL ETHANOL EXPOSURE ON CHOLINERGIC DEVELOPMENT IN THE SEPTOHIPPOCAMPAL SYSTEM OF THE RAT. D.J. Swanson, D.D. Walker, and M.B. Heggen*, University of Florida College of Medicine and V.A. Medical Center Gainesville, FL, 32610.

In animal models of Fetal Alcohol Syndrome (FAS) the hippocampus has been shown to be especially sensitive to the effects of prenatal ethanol exposure, exhibiting neuronal loss and alterations in neurotrophic factor elaboration. We have begun to characterize the influence of chronic prenatal ethanol exposure on the development of the cholinergic neuronal population which projects to the hippocampus, the medial septal nucleus and nucleus of the diagonal band (MS/DB). On gestation days 12 pregnant dams were either fed an ethanol containing liquid diet, pair-fed a calorically equivalent sucrose containing diet, or given rat chow ad lib. Preliminary evidence shows that chronic ethanol exposure produces a significant decrease in MS/DB ChAT activity at postnatal day 1 (P1; 18% reduction in ethanol exposed pups compared to pair-fed pups). Subsequently, at P7 ChAT activity expression in ethanol exposed pups approximately equaled that of pair-fed and chow control pups. Further experiments are in progress to examine additional developmental time points as well as the anatomical basis for this alteration in ChAT developmental expression. These studies will determine whether the early decline results from a developmental delay, or whether a reduction in cholinergic neurons coupled with up-regulation of ChAT expression may occur. (Supported by AA05332, AA02000, a grant from the A.B.M.R.F., and the Dept. of Veterans Affairs.)

648.9

ETHANOL EXPOSURE INCREASES GFAP mRNA AND PROTEIN IN DEVELOPING RAT CORTEX AND CULTURED CORTICAL ASTROCYTES. T.L. Fletcher* and W. Shan, School of Public Health, The University at Albany and Wadsworth Center, Albany, NY 12201.

Prenatal exposure to ethanol can result in Fetal Alcohol Syndrome (FAS). We have shown that brief ethanol exposure causes changes in expression of a limited number of genes in the developing rat CNS. We describe here ethanol's effect on glial fibrillary acidic protein (GFAP) expression. Artificially-reared rats pups received 3.3 g/kg/day from postnatal days 5-7 resulting in peak blood alcohol levels of 180 mg/dl. Artificially-reared control pups received an isocaloric diet. Suckling control pups were reared by their dams. Two hours after the last dose of ethanol, mRNA was isolated from three brain regions of some pups. Other pups were fostered to control dams and mRNA was isolated at 14, 21, or 90 days of age. Northern and slot blot analyses demonstrated a transient 3-fold increase in GFAP, but not β-actin, mRNA in cortex. No changes in GFAP were observed in the hippocampus. Western blot analysis of 7 day cortex showed a comparable increase in GFAP protein. To determine if this increase was due to a direct action of ethanol on glial cells, primary cultures of cortical astrocytes were exposed to ethanol exposure in media containing concentrations equivalent to pup ethanol exposure. Ethanol exposure increased GFAP expression parallel to the in vivo observations. These results suggest that ethanol directly disrupts the regulation of specific genes in astrocytes during CNS development. (Supported by AA-07472)

648.10

THE EFFECT OF EARLY POSTNATAL ETHANOL EXPOSURE ON LIGHT-DARK PREFERENCE IN PREWEANING RATS. Jamie H. Wilson* and Sandra J. Kelly. Department of Psychology, University of South Carolina, Columbia, SC 29008.

This experiment examined the influence of early postnatal ethanol exposure on light-dark preference and the effect of home-cage shavings versus clean shavings on this preference. Rats were artificially reared and exposed to either 3 or 5 g/kg/day of ethanol from postnatal days 4 through 10. Control groups consisted of rats artificially reared but not exposed to alcohol and rats normally reared by dams. All rats were reared by dams from postnatal day 12 to the time of testing on postnatal day 16. The testing apparatus consisted of two chambers: one was made of black Plexiglas and completely enclosed and the other was made of white Plexiglas and open to bright lighting. A small door connected the two chambers. Each animal was placed in the black chamber facing away from the dark chamber. Latency to enter the dark chamber and total time spent there were recorded.

The presence of home-cage shavings resulted in more time spent in the white chamber. It is plausible that the presence of home-cage shavings reduced the stress associated with a novel environment such that the animal leaves the shelter of the black chamber. There was a distinct sex effect in that males spent more time in the dark chamber than females. High-dose and low-dose animals spent less time in the dark chamber than animals reared by dams. Both control groups spent the same amount of time in the dark chamber. Early postnatal exposure to ethanol may cause a decrease in the adaptive alternative explanation is that there was an increase in exploratory behavior due to ethanol during development. (Supported by NIAAA Grant AA08080 to S.J.K.)
470.11 AUDITORY BRAINSTEM RESPONSES (ABR) IN RATS PRENATALLY EXPOSED TO ALCOHOL: EFFECTS OF CRITICAL PERIODS. M.W. Church*, G.W. Overbeck, P. Holmes, J. Tilak. Fetal Alcohol Research Center, Dept. Ob/Gyn, Wayne State University School of Medicine, Detroit, MI 48201.

Prenatal alcohol exposure can cause sensory disorders and other nervous system morbidity. Our interest concerns the auditory system. For example, children with the Fetal Alcohol Syndrome (FAS) have a variety of hearing disorders (Church & Garkin, Pediatrics 82:147-154, 1988; Church & Eldis, Alc. Clin. Exp. Res. 16:380, 1992). Using an animal model, we have also observed congenital hearing loss (Church, Alcohol 4:231-239, 1987) and developmental delays in auditory maturation (Church & Holloway, Alc. Clin. Exp. Res. 8:258-261, 1984) as evidenced by the ABR. The degree and type of morbidity caused by prenatal drug exposure depends, in part, on the gestational age at the time of exposure. To study the effects of "critical periods" of exposure, Sprague-Dawley rats were prenatally exposed to alcohol by administering liquid diets containing either 0% or 35% ethanol-derived calories to pregnant dams from gestation day 7-14 or 15-22. Untreated control groups were also used. The earlier (organogenesis) period of exposure proved more critical in producing ABR maturation delays in the offspring than the latter (histogenesis) period. Supported by NIAAA Grant AA07606.

471.1 GLIA AND OTHER NON-NEURONAL CELLS IV


Cervical spinal cords from premature infants were obtained at autopsy and immersion fixed in Zamboni's fixative. Transverse 60μm vibratome sections were cut and stained with antibodies to human neuron, chondroitin-4-sulfate (CS-4), vimentin (VIM) or glial fibrillary acidic protein (GFAP) using a biotin-avidin-peroxidase protocol.

In developing white matter, radially-oriented astrocytes are both GFAP and VIM positive before 33 weeks of gestation. Few satellite astrocytes are evident in the white matter prior to that time. Later, the predominant white matter astrocytic type is tall and appears to express GFAP only. The distribution and density of these cells correlate with the degree of myelination. GFAP-positive astrocytes are evident in the gray matter at 28 weeks, but are more numerous and more ramified by 33 weeks.

CS-4 is distributed throughout white and gray matters at the gestational ages examined. In the regions of the unmyelinated corticospinal tract, CS-4 Immunoreactivity is associated with septae. These septae are also GFAP and VIM positive prior to 33 weeks of gestation, but only GFAP positive at later times.


We have previously demonstrated that embryonic neurons growing in a substrate ofmitocerebral cortical astrocytes were selectively labeled with the vital dye 5(6)-carboxy fluorescein diacetate (CFDA). Neurons displayed brilliant green somata as well as intensely labeled processes while the glial monolayer showed only a dim level of fluorescence. Using optical recording methods, we demonstrated that both neurons and astrocytes hydrolyze CFDA to the fluorescent free acid. However, once CFDA is removed from the medium, the astrocytes rapidly expel the dye with a time course of minutes as opposed to neurons which retain the dye for several hours. This has allowed the selective labelling of neurons with CFDA. When astrocytes are rinsed at 4°C, they retain the dye for several hours, suggesting that an active pump is responsible for the exclusion of the dye. The exclusion of the dye is also inhibited by reserpine in a concentration dependent fashion. Immature astrocytes were not able to expel the dye. However, the ability of embryonic astrocytes to pump fluorescent dyes develops during the first week in vitro. Moreover, the immortalized V1 cell line from embryonic mouse hypothalamus, which expresses several mature astroglial properties, is able to expel the dye. This cell line will be used to investigate the properties of this membrane transporter. Supported by NIH NS 24168.

471.3 NEURONS FAIL TO INDUCE BRAINSTEM ASTROGLIAL CELL DIFFERENTIATION IN VITRO. D.L. Cooper and M.E. Hamburger. Department of Psychology and Center for Neurobiology and Behavior, Columbia University, New York, NY 10032.

Tumors derived from cells of astrocytic lineage are the most common and devastating of the mammalian brain. Previous in vivo analyses, in which cerebellar astroglia and granule neurons were purified from early postnatal cerebellum and combined, demonstrated that neurons provide a control mechanism for astroglial growth, inducing cerebellar astroglial glial differentiation via an extracellular neurotrophic activation of TGFβ. In the present study, we examined whether astroglial cells from the brainstem, a more primitive brain region where neuronal layers do not form, respond to neuronal regulatory signals and TGFβ-induction of glial differentiation.

When neurons, purified from either brainstem or cerebellum, are co-cultured with brainstem astroglial cells, the neurons bind poorly to the astroglial cells and do not induce glial differentiation. Although neurons do not induce brainstem astroglial differentiation, brainstem glia had an augmented response to the TGFβ. In the absence of neurons or TGFβ, brainstem astroglia showed a 2-3 times higher than that of cerebellar astroglial DNA synthesis. Addition of TGFβ to 85 or 1(10 ng/ml) resulted in a 50-60% reduction in DNA synthesis in brainstem glia as compared to the 26-61% reduction seen with cerebellar astrocytes. At similar concentrations, both TGFβ induced glial differentiation, measured by Northern blot analysis of GFAP expression and the glial fibrillary acidic protein (GFAP) expression.

These experiments demonstrate the ability of TGFβ, but not neurons, to induce differentiation of brainstem glia, and suggest that the failure of neurons to induce differentiation may be related to neuron-glial binding.


The studies reported here were aimed at establishing an antigenic marker specific for olfactory nerve glia throughout development. We have recently developed a monoclonal antibody (I58) that recognizes both myelinating and non-myelinating Schwann cells in the chick (Bhattarcharya, et al. 1991) but does not recognize astrocytes, oligodendrocytes or neurons. In the current study, we used the I58 antibody and the avidin-biotin-HRP technique (Vectastain) on tissue sections of embryonic and adult chickens. I58 immunostained olfactory nerve glia both in early embryos and in adults indicating that olfactory nerve glia may be more closely related to non-myelinating Schwann cells than other glial cells types. Further, the pattern of immunostaining we obtained is consistent with the idea that most, if not all, progenitors of olfactory glia are derived from the olfactory placode rather than the neural crest. We also observed that immunostained glia were initially distributed along the perimeter of the olfactory nerve (late E4). By the end of E5, I58-immunostaining was found throughout the nerve. Finally, glia appear to enter the olfactory bulb with the olfactory nerve. In the adult, a thin rim of I58 immunopositive cells can be observed around the edge of the olfactory nerve layer. This work was supported by NIH grants NS30047 (RBN) and NS27277 (RB and NR).

X-irradiation is a useful tool for manipulating the cellular composition of an organ and exploring the developmental consequences. Exposure of the spinal cord to a single 3-day-old x-ray exposure induces a profound and incomplete depletion of astrocytes and oligodendrocytes (J. Neupath. Exp. Neurol., 22:294-301, 1963). Exp. Brain Res. 75:513-522, 1989). This in vitro study addresses issues related to the vulnerability of specific cell phenotypes and maturation of normal embryonic spinal cord of rats were irradiated and removed within one hour to establish the cultures. Non-irradiated spinal cord from littermates provided the control cultures. Cells were cultivated for 8 days in serum-free medium. Immunohistochemical markers were used to characterize the cultures. In cultures derived from x-irradiated tissue, growth was markedly diminished and a significant portion of the surviving cells were GFAP-positive astrocytes. These cells were either primary or epithelial, and some were colocalized in the majority of the epithelial cells. Galactocerebroside, a marker for mature oligodendrocytes, was immuno-localized in an abundant population within control cultures but was virtually absent in cultures from x-irradiated rats. However, a small population within cultures from x-irradiated rats exhibited immunolocalization of A2B5 and 2,3-CNP, markers of early oligodendrocytic lineage. This primary study demonstrated reduced survival and viability of glia in cultures derived from x-irradiated spinal cord. Moreover, the absence of mature oligodendrocytes suggested a specific vulnerability of a stage in this cell lineage.

Supported by NIH Grant NS 04761.

IMMUNOCYTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERIZATION OF GLIAL CELLS IN LONG-TERM CO-CULTURES. C. Andersson, J. Brunsle-Behnold, and M. Tytell. Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27101.

We examined a mixed glial culture containing type 1 astrocytes and 0-2A lineage cells in fetal calf serum at 5 days in vitro (DIV). 12 DIV, and 30 DIV, using cell-specific immunocytochemical markers and electron microscopy. The flat type 1 astrocytes were polygonal, and GFAP+, GalC- and A2B5- (specific markers for natural astrocytes and oligodendrocytes, and 0-2A cells) at all three time points. From 5 to 30 DIV, the type 1 astrocytes increased in cell size. Ultrastructurally, the stochastically processed axons dramatically changed over time, with the numbers of glial filaments increasing and microtubules decreasing. At 5, 12, and 30 DIV, the 0-2A lineage cells were multipolar, and A2B5+, GFAP-, and GalC-. Ultrastructurally, the 0-2A lineage cells could not be regarded as either astrocytes or oligodendrocytes. These cells had a dense cytoplasma, a very small number of intermediate filaments, and a large number of vacuoles and dense bodies. At no time were the differentiated type 1 astrocytes immunoreactive for HNK-1 and NCAM. 0-2A lineage cells were HNK-1- and NCAM-, further suggesting cellular immaturity.

The cytoskeleton of cultured type 1 astrocytes seems to develop comparable to astrocytes in vivo. Under these culture conditions, 0-2A lineage cells were multipolar, but immature, and appear unable to differentiate.

INSULIN-LIKE GROWTH FACTOR 1 (IGF-I) INDUCES ASTROCYTES TO ACQUIRE A RADIAL GLIAL-LIKE MORPHOLOGY IN VITRO. A. Casey and B.J. Duff. Dept. of Anatomy, University of Saskatchewan, Saskatoon, SK, Canada S7N 0W0.

Insulin and IGF-I are known to have neuroprotective effects; however, they can also affect astrocyte function. For example, Dugan and Blankenhorn have shown that they both affect glycogen metabolism (1992, J. Neurounch. Sci. 53:311) and have shown that they affect astrocyte neuroprosthetic activity (Ang et al. 1992, J. Neuroi. Sci. in press). Since astrocyte morphology is independent of astrocyte function, our objectives were to determine whether insulin, and in particular IGF-I affected astrocyte morphology. Rat type 1 astrocytes were isolated using the McCarthy and de Vellis method. After two weeks in a serum-containing medium, the cultures were maintained on Bottenstein's G5 medium minus the insulin. In this medium the astrocytes maintained their flat fibroblast-like appearance. Treatment of insulin (1-5 ng/ml) or IGF-I (10-100 ng/ml) resulted in a dramatic change in astrocyte morphology. In contrast, IGF-II (10-100 ng/ml) had no effect on astrocyte morphology. The insulin and IGF-I induced morphology consisted of a distinct soma from which one emerged long prominent and sometimes several long processes of narrower caliber. The processes were tightly packed with GFAP-containing intermediate filaments, whereas the soma had a lesser density of intermediate filaments. This morphology is very reminiscent of that of radial glial cells. In the CNS, IGF-I mRNA as well as IGF-I receptors are expressed by both neurons and astrocytes in a stage-specific manner, i.e. the highest level of IGF-I expression occurring in embryonic and fetal development. It is likely, therefore, that IGF-I can act in a permissive as well as an autocrine fashion in the brain. We postulate that possibly IGF-I plays a role in the differentiation of radial glial cells. Furthermore, these packets and/or autocrine effects of IGF-I may be involved in the relationship of developing neurons and radial glia, which affects both neuronal migration and the differentiation of neurons and astrocyte. We thank Dr. B. Bhaumik for the gift of IGF-I and IGF-II.

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Glia and Other Non-neuronal Cells IV

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1011.0 THE ISOLATION OF A NOVEL OLIGODENDROCYTE SPECIFIC c-DNA
C. Schefer, N. Schenaer-Wenzler, C. Decaner, and M. E. Schwab.

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Myelin contains a variety of unique proteins (e.g. MAG, MBP, PLP, CNPase). Only a few proteins seem to be selectively expressed by central nervous system (CNS) oligodendrocytes as compared to peripheral nerve Schwann cells. Myelin disorders that are specific for the CNS exist, however, and oligodendrocytes play important additional roles in CNS development and regeneration by their expression of neurite growth inhibitory proteins. Here we demonstrate the isolation of a c-DNA encoding a novel oligodendrocyte specific clone. The c-DNA corresponds to a c-RNA of 4.9 kb. The presently available c-DNA (3.4 kb), including the poly-A tail, does not show homology to any known oligodendrocyte or CNS c-DNA. The expression is restricted to the postnatal CNS. The encoded protein is expressed in astrocytes (PNS), liver, lung, thymus, heart, kidney, spleen, skeletal muscle, testis, and is higher at P10-20 than in the adult spinal cord. In situ hybridisation shows a stronger expression in oligoden- drocytes in myelinating regions of the brainstem, cerebellar white matter and corpus callosum. In cultured optic nerve oligoden- drocytes P111 expression was localized in galC-positive oligoden- drocytes.

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471.11 THE GLIOARCHITECTONICS OF THE HUMAN NEOCORTEX. T.J. Mandryk. Department of Pathology and Laboratory Medicine, University of Cincinnati and Laboratory, Cincinnati, OH 45267-0529.

It is well known that the normal cortex shows few fibrillary astrocytic fibers. In the molecular layer of these areas, the astrocytes are associated with external glial limiting laminae. In the disease process in Alzheimer's disease of the molecular layer. This might be associated with gliosis, but in the aged individual, and in Alzheimer's disease, the astrocyte's molecular layer displays fibrillary fibers which penetrate down to the II and III layers. The astrocytes in the molecular layer are also seen in the thalamus, cortex, and thalamus.

471.12 MULLER CELL PHENOTYPE PRECEDES SENEQUENCE OF VIRALLY TRANSFORMED CHICK NEURORETINAL CELLS. G.M. Siegel, E.L. Emperador, J.T. Hansen, and M.F.D. Notter. Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

The phenomenon of senescence has been an obstacle in obtaining permanent Rou's sarcoma virus-transformed avian cell lines. Our previous studies analyzed this senescence phenomenon in Rou5 sarcoma virus-transformed chick neuroretinal cells (L29NR) and demonstrated a decreased mitotic index accompanied by loss of transformed phenotype (Siegel and Notter, submitted, JVI 384-92). After four to five months in culture, new cell death, unusual process formation is observed in L29NR cell populations. We have now characterized these morphologically differentiated cells as immunoreactive for glial fibrillary acidic protein, S-100 antigen, and vimentin, while predominantly negative for neuron-specific enolase, as detected immunocytochemically. Electron microscopic observations revealed large, vacuolated cells packed with longitudivally-oriented intermediate filaments, extensive smooth and rough endoplasmic reticulum, as well as occasional myelin-like figures, characteristic of normal retinal Muller cells. However, unlike normal Muller cells, retinal Muller cells have been described to be intracerebrally and extracellularly. From these data, we conclude that despite continued retroviral expression, senescence L29NR cells exhibit a Muller cell phenotype as a state of terminal differentiation at the end of their mitotic lifespan in culture.

This work was supported, in part, by T32AG00107 (G.M.S.), EY06947 (M.F.D.N.) and NS25778 (J.T.H.).


During metamorphosis in Prosopha, the central nervous system (CNS) is extensively remodelled. Adult nerves arise from the embryonic glial layer and in the nerve formation is extensive pathfinding. Some glial cells may serve as landmarks to define pathways for developing neurites. At the boundary between the nerve and cortex, unique glia cells have an affinity for Notch antibodies (Ab) in third instar larvae (Pellon et al., J. Cell Biol. 113:657). In early pupae, Notch Ab affinity is most prevalent in the glial cells at the neurite where the neurites are forming. By 48h, the affinity decreases and is barely evident at 72h; this period is coincident with final leg nerve formation. Related miotic activity, typical for many other Notch-positive cells, is absent in these glia. These neurone glial cells are distinguished by intense synthetic activity in parallel with the peak Notch affinity (48h pupae), as indicated by extensive rough endoplasmic reticulum.


In this study we have modified techniques for culturing neurons of pulmonate molluscs to obtain populations of cells having glial-like characteristics. The cells were isolated from the cortical regions of the central ganglions of snails Lymnaea stagnalis and Helisoma trivolvus using protease (see Ridgway et al., J. Neurobiol. 22: 377-390, 1991). The isolated cells were then cultured under sterile conditions in 36C Leibowitz (L-15) medium on various substrates. The putative glial cells displayed an elongate morphology (75-100 μm in length and 10-15 μm in width), the nucleus being situated midway along the cell axis. Lamellipodial processes extending from the perimeter of the cells correlated with a high degree of motility in the first 6-12 h after plating. This motility was substrate specific: on adhesive substrates (e.g., poly-L-lysine) cells decreased in motility after 12 h, whereas those plated on less adhesive substrates (e.g., bovine serum albumen) were motile for 48-72 h. Electrophysiological recordings made from motile cells revealed resting membrane potentials ranging from -40 mV to -50 mV. The cells were responsive to both dopamine (0.1-1.0 μM) and noradrenaline (0.1-0.1 μM). Cells could be maintained for 4-5 days whereupon they became pycnotic and lost their membrane potentials; reasons for this cell death are under study.

While early animal models of schizophrenia addressed primary changes in striato-limbic dopamine activity, they did not account for other phenomena associated with the illness (e.g. congential temporal-limbic abnormalities, cortical deficits, vulnerability to stress, postpubertal onset). We have reported that ionotonic acid ventral hippocampal (vH) lesions in adult rats affected DA-related behaviors in a manner consistent with increased mesolimbic DA activity. These changes were paralleled by biochemical indices suggesting enhanced DA transmission in the nucleus accumbens, a reduction in the medial prefrontal cortex, and an increase in corticosterone levels in hippocampal CA1 (Brain Res., 1992). The behavioral response to pharmacological (FG-7142) or environmental (swim stress) stressors was not affected. In contrast, locomotor activity and response to amphetamine of rats lesioned as neonates (PD7) did not differ from controls 4 weeks after the lesion (PD35). However, 3 weeks later (PD56), the same rats became hyperactive in these conditions. Moreover, neonatal lesioned animals were hyperactive in stress, in contrast to rats with neonatal lesions of VH, augmented activity developed only after puberty. Neonatal but not adult-induced lesions also produce an exaggerated response to stress, with increased serum corticosterone levels.

Homologous mechanisms could be involved in schizophrenia.

471.2 DEVELOPMENTAL EXPRESSION OF PARVALBUMIN mRNA IN THE CEREBRAL CORTEX AND HIPPOCAMPUS OF RAT. Leena Luu de*, Eduardo Soriano*, Jose A. del Rio, Sonia Formigari**, University of Cordoba, Dept of Neurobiology, 14004 Cordoba, Spain.

Parvalbumin is a calcium binding protein that is thought to play a major role in calcium buffering in metabolically active fast-firing cells. In adult cortex, parvalbumin is expressed in a subset of cholinergic GABAergic interneurons. The pattern of parvalbumin mRNA expression during the postnatal development of rat cerebral cortex and hippocampus was examined by means of in situ hybridization with an oligonucleotide probe. In animals aged P0-P14, no signals of expression were detected in the adult cortex and hippocampus, with the exception of a few low numbers of motor neurons in motor and somatosensory cortices and in the hippocampus. By P12, parvalbumin expressing cells were detected within all cortical regions. At P14 an overall increase both in the number of positive cells and in the intensity of labeling was observed. A further maturation pattern was seen at P16-P21 stages, which lead to the appearance of an adult like distribution in cortex and hippocampus. The appearance of parvalbumin mRNA expressing cells does not follow the usual inside out sequence of cortical maturation. Instead, hybridization is first observed in the middle cortical layers to thereafter expand to the immediate adjacent layers. Count of silver grains revealed that hybridization signals increased progressively during postnatal development. In adult neocortex and hippocampus, the distribution of parvalbumin mRNA containing cells is consistent with our previous immunocytochemical findings in rodent cortex. These data suggest that the developmental pattern of expression of parvalbumin is similar to that of calcium buffering and may reflect the functional maturation of cortical interneurons.


The neurological deficits found in infantile hydrocephalus have most often been explained by pathological changes in cerebral cortex. It has been the primary objective of our studies to discover a cellular basis for these residual neurological deficits observed even though surgical intervention may have relieved the effects of ventriculomegaly on the cerebral cortex (McAlister et al., 1980). We have shown significant structural changes in cerebral cortex, basal forebrain region, especially in septal nuclei, and hippocampus. A primary difference in the structural alterations within these regions was the minimal increase in extracellular space in the hippocampus. In the present study we compared the ependymal surface adjacent to the cerebral cortex, basal forebrain region, and hippocampus. Kaolin injection induced hydrocephalus; aldehyde fixed sections were stained coronally with subsequent staining for cells and fibers. The ependyma underlying the cerebral cortex was thinned but appeared to remain intact. In contrast, the ependyma adjacent to the hippocampus was not altered in comparison to control tissues. The cuboidal shape as well as surface projections appeared normal. The potential relation of these structural differences to extracellular space in the neuropil is discussed. Supported by AA091031,RMK-NH/HD215572, JPM.

471.4 CYTOARCHITECTURAL, NEUROREL MOPHOL O GIC AND MOLECULAR ASPECTS OF HUMAN HIPPOCAMPAL DEVELOPMENT. Steven E. Arnold* and John D. Trojanowski, Departments of Psychiatry and Pathology, University of Pennsylvania School of Medicine.

Cytoarchitecture, neuronal morphology and the expression of various neuronal cytoskeletal proteins were examined in human hippocampal fixed sections from the hippocampal formations of 18 cases 10 weeks gestational age through 2 years were stained with cresyl violet for cytoarchitecture and immunophenotypic analysis and processed for immunohistochemistry. Monoclonal antibodies (mAb) used were directed at components of the neuronal cytoskeleton that are believed to be important determinants of neuronal polarity. These included microtubule-associated proteins (MAPs), MAP2, MAP3 and tau, alpha and beta tubulins, and poorly, moderately and highly phosphorylated MAPs. Immunostaining was performed on sections from five human fetal brains. Differential patterns of cytoarchitecture and neuronal morphology were noted across time and both between and within anatomic regions. The expression of MAP2, MAP3, alpha and beta tubulins, MAPs, and MAPs was evident at the earliest time point studied and persisted throughout development. Immunoreactivity to tau and NFP-F-* appeared at subsequent points, with tau diminishing in intensity later on in development. MAP2, MAP3 and NFP-F immunoreactivity allowed recognition of nascent dendrites and pyramidal shaped neurons prior to their definition with conventional cresyl violet staining. At all times, there were differences in intensity of immunoreactivity between the different amaminergic, subicular and entorhinal subfields. (Supported by NIH grant 1 R01 MH09708-01)

471.5 CALBINDIN-D28K DISTRIBUTION IN HIPPOCAMPAL FORMATION OF LATE GESTATION FETAL SHEEP IS INDEPENDENT OF PLASMA GLUCOCORTICOID CONCENTRATIONS. T.J. McDonald & R.H. Wasserman, Department of Physiology, Cornell University, Veterinary Research Tower, Ithaca, NY 14850.

Glucocorticoids regulate the appearance of Calbindin-D28K in the granule cells of the dentate gyrus of adult rats with adrenalcausing complete loss of immunoreactivity in the granule cell layer after 4 weeks (Jacopo and Rosado, 1990; Aronson et al., 1980). In late gestation, fetal sheep undergo a logarithmic rise in peripheral plasma cortisol concentration that starts at approximately 125 days of gestational age (DGA) with baseline concentrations of 10-15 ng/ml peak at term with concentrations over 100 ng/ml" (Magee et al. 1981. J.Ster.Biol: 14:1091-1099). This cortisol rise is driven by the pituitary and hypothalamic paraventricular nuclei and is indispensable for parturition to occur at term (Liggin et al., 1979; Recq et al., 1981; 1983; McNamara & Nalaniek, 1991. Am.J.Obstet.Gynecol: 165:764-770). In this study we analyzed the fetal sheep at 105, 125, 135, 147 (in labor) DGA and newborn lambs were examined immunocytochemically. While Calbindin-D28K was found in diverse areas of the brain, e.g. brainstem, cerebellum, neocortex, basal ganglia and hypothalamus, no Calbindin-D28K immunoreactivity was detected in the dentate gyrus or Ammon's horns at the ages examined. However, many Calbindin-D28K immunoreactive cells were found in the subiculum at all ages, but intensity of staining appeared to be independent of gestational age. It is concluded that unlike in adult rats, abstract image glucocorticoids have no effect on the weaner immunocytochemical distribution of Calbindin-D28K in the hippocampal formation of the late gestation fetal sheep.

471.6 NEUROGENESIS OF THE VASOPRESSIN NEURONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS AND MEDIAL AMYGDALOID NUCLEUS OF THE RAT. H.A. Al-Shammari & G.J. De Vries, Program in Neuroscience and Behavior, Univ. of Massachusetts, Amherst, MA 01003.

The vasopressin-immunoreactive (AVP-ir) neurons of the bed nucleus of the stria terminalis (BST) and medial amygdaloid nucleus (MA) share many neurochemical and anatomical characteristics, e.g., in their neurotransmitter content, cell morphology, and steroid sensitivity. It is unclear, however, how BST and MA neurons develop these similar characteristics. To get more information about the development of these cells, we determined the day of birth of these AVP-ir subgroups with the cell birth marker bromo-2-deoxy-5-uridine (BrdU).

Pregnant Long-Evans rats received intraperinatal injections of BrdU on one of gestational days 14-20, day 1 being the day that a copulatory plug is found. At three months of age, the male offspring of these treated females were sacrificed and their brains were processed for both BrdU and AVP immunostaining. Approximately 20% of the cells in the BST and MA were also immunoreactive for BrdU, the majority of which were found in the BST and MA of animals exposed to the cell birth marker on embryonic day 16. In this study, the preliminary findings are in agreement with earlier studies using [H]thymidine autoradiography which suggest that most of the cells in the divisions of the BST and MA that contain the AVP-ir neurons are born on embryonic days 15-16 and 14-15, respectively.
472.7

Zones containing post-mitotic cells and neurons forming neurites express proto-oncogenes. The change in the expression of c-fos, c-neu, c-sis, and c-ras, was examined using immunobots. Tissue from the frontal pole of rat cortex was harvested at various ages (between gestational day 16 and postnatal day 52) and in 3- to 5-month-old rats. Blocks of tissue were disrupted in buffer containing detergent and proteinase inhibitors. Standard gel electrophoresis and transfer techniques were used in probing for immunobots. Anti-c-fos identified 2 proteins, one with a molecular weight of 64 Kd and the other 80 Kd. c-fos expression of both proteins appeared on G16 and was peaked at P5 and on G18. Anti-c-neu also labels 2 proteins, 65 Kd and about 185 Kd. The temporal expression of the 65 Kd protein was similar to that of c-fos. On the other hand, the 185 Kd protein appeared on the day of birth (P0) and virtually disappeared by P8; peak expression occurring on P3-P6. Anti-c-sis identifies a doublet with molecular weights of 53 and 62 Kd. The expression of both proteins begins as early as G16 and persists beyond P9. This expression was rather stable throughout this period. Trace amounts of all of these proto-oncogenes were detectable in the adult rat. The pattern of c-ras expression is different for it builds during the postnatal period to adult levels by P21. Based upon timing, the data are consistent with the concept of a cascade of events, c-neu being related to cell proliferation or neuronal migration, c-neu and c-fos being related to neuronal differentiation and or death, and c-ras being related to neurite outgrowth, synaptogenesis, and the maintenance of neuronal integrity. Funded by DE 07734, AA 06916, and AA 07568.

472.8
MIGRATION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IMMUNOREACTIVE NEURONS TO RAT CORTEX. M.W. Miller*, Res. Serv., Iowa City V.A.M.C., and Dept. Psychiatry & Pharmacology, Univ. Iowa Coll. Med., Iowa City IA 52242.

Most neurons migrate to cortex from the transform inside-to- outside sequence. Accordingly, neurons in deep cortex are generated before those in superficial cortex. In visual cortex, this migrational pattern is followed by cortico-cortical projection neurons and by GABAergic local circuit neurons. A combined autoradiographic-immunohistochemical method was used to determine the time of origin of VIP immunoreactive neurons. VIP is expressed by a subpopulation of local circuit neurons, mostly small bipolar neurons, which do not co-localize GABA, nor of neurons in a layer of cingulate cortex (areas 24 and 29), somatosensory cortex (area 3), and visual cortex (area 17) was examined by administering [3H]thymidine on gestational day 13, 15, 17, 19, or 21 and sacrificing the pups on postnatal day 30. Although the timing differed slightly in each cytoarchitectonic area, the basic pattern of neuronogenesis was similar in all areas. VIP-positive neurons with a particular time of origin were not distributed in a tangential band of cortex; rather such neurons were distributed through the full depth of cortex. Such a pattern was evident regardless of the time of origin. Thus, VIP immunoreactive neurons migrate into cortex via a mode other than the standard inside-to-outside sequence. Funded by DE 07734, AA 06916, and AA 07568.

472.9
POSTNATAL DEVELOPMENT OF PROTEIN KINASE C GAMMA EXPRESSION IN RAT BRAIN. R. Burwida, C. Nyakas, E.A. van der Zee, S. Cazabon, P.M. Luiten*. Dept. of Animal Physiology, University of Groningen, PO Box 74, 9700 AA, The Netherlands.

Findings of sexual dimorphism in the rat corpus callosum, using gross size measures, prompted the use of electron microscopic techniques to view ultrastructural parameters. Since neonatal handling was found to enhance certain differences between gross measures of female and male rat callosa, both handled and nonhandled rats were used to study this phenomenon. All rats were transcerebrally perfused at 110 days of age, and mid sagittal sections were obtained. Based on previous findings, sampling was restricted to the first 20% of the genu, although the posterior portions of the callosa have been retained and embedded for possible analysis. Preliminary findings indicate a greater quantity of unmyelinated axons than myelinated axons in the areas sampled, and a distinct Sex x Handling interaction in total number of unmyelinated axons.

472.10

472.11

A novel Antp-class homebox gene has been isolated from a human 11 W fetal brain cDNA library. PCR with two sets of oligonucleotide primers (specific for highly conserved regions of the Antp-class homeobox) was used to amplify portions of the gene present in the fetal brain library. Sequencing 100 clones identified 2 novel Antp-class genes. Screening the fetal brain library for a probe specific for one of the novel genes led to the identification of a 1.7 kb cDNA containing the novel homeobox sequence. This clone encodes a protein of approximately 327 amino acid residues. By Northern analysis, this cDNA detects multiple transcripts in the developing human CNS as well as other tissues. These observations suggest that in early mammalian development this homebox gene may act as a spectrum of control functions in a variety of organ systems including the CNS.

472.12

We introduce a developmental neuronalontological database from J.L. Cohen as a potentially useful framework for computational models and analysis of the developing human brain to 6 years and its relation to developing behaviors. We have investigated the relation between functions related to gyral and anatomical measures of cortical thickness, cell density, cell body length, cell body width, f of axons/ unit area across all layers and over 40 gyri. Using the analytical method, Optimal Multi layer hidden layer network, inputs consisting of the 6 variables per gyrus per layer were used to find the best separation of the gyral functions used the diverse variables. We wished to see which variables, layers and gyri maped to gyral functions found at least similar possible. We wished to see which variables, layers and gyri mapped to gyral functions found at least similar possible. Results will be presented. Also presented are data on total critical thickness, figure 1 graphs the total cortical thickness in mm., for the 37 loci for all eight age-groups in this age range. The correlation is not smooth, with many loci showing episodes of reduction in cortical thickness despite the general tendency to increased thickness with age. Figure 2 graphs the slope rates of change in cortical thickness per mm. per month of the values for total cortical thickness, for the seven age-blocks the graph showing that all loci show appreciable change in cortical thickness, either positive (increasing to thickness) or negative (decreasing to thickness) over the age-block 0-1 month, with the rate of change of total cortical thickness per month generally decreasing with age.
473.1

CHOLINE ACETYLTRANSFERASE IN THE CEREBRAL CORTEX OF NBM-LESIONED RATS RECEIVING CHROMAFFIN CELL GRAFTS. S. A. Weiner* and Z. C. Koyt. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal, Quebec, Canada H4H 1R3.

Lesions of the nucleus basalis magnocellularis (NBM) in rats produce spatial memory deficits as well as changes to cholinergic markers in the cerebral cortex, the target area of projections from the NBM. We have previously reported that adrenal chromaffin cells transplanted to the cerebral cortex of rats with NBM lesions are able to ameliorate lesion-induced spatial memory deficits and increase acetylcholinesterase (AChE) staining in the host cortex (Weiner et al., Brain Research, 527: 163, 1990). In the present experiment, we tested the hypothesis that grafting chromaffin cells to the cerebral cortex of NBM-lesioned rats will have an effect on a different cholinergic marker in the cerebral cortex, choline acetyltransferase (ChAT). At various time points post-grafting, Sprague-Dawley rats received bilateral partial lesions in the NBM and, two weeks post-lesion, either were left unoperated or had suspensions of adrenal chromaffin cells grafted to six sites in their frontal and parietal cortices. A group of unoperated rats served as controls. ChAT activity was measured at 2, 6, 12 and 16 weeks post-graft. In the lesioned-alone group it was found that ChAT progressively decreased over time, whereas in the lesion plus graft group, ChAT levels were significantly decreased at 6 weeks and appeared to be increasing at the 11 and 16 week time points. These results support previous findings that the cholinergic system of cerebral cortex following grafting of chromaffin cells to the cortex of NBM-lesioned rats is increased. (Medical Research Council, Canada & Fonds de la Recherche en Santé, Québec.)

473.2

FOCAL FIBROBLAST GROWTH FACTOR IN CHROMAFFIN CELL GRAFTS TO THE CEREBRAL CORTEX OF NBM-LESIONED RATS. E. S. Zucol, Z. C. Koyt and S. A. Weiner. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal, Quebec, Canada H4H 1R3.

Chromaffin cell grafts to the frontal and parietal cortices of nucleus basalis magnocellularis (NBM)-lesioned rats can ameliorate the spatial memory deficits which result from the lesions; this is accompanied by an increase in staining for a cholinergic marker in the host cerebral cortex (Weiner et al., Brain Research, 527: 163, 1990). Lesion of chromaffin cells of the adrenomedulla are known to contain basic fibroblast growth factor (bFGF), a known neuroprotectant, it is of interest to determine whether bFGF may be involved in the grafted effect. As a first step, it is necessary to test whether bFGF is present in these grafts at various time points post-grafting. Taz-mained rats were unoperated and then administered bFGF as a control or chromaffin cell grafts, two weeks post-lesioning. Rats were retstoned on the Taz-mate at various time points post-graft; subsequently, the density and distribution of bFGF in grafts and the area surrounding the grafts was measured using immunocytochemical techniques. Whereas the host cortex is virtually devoid of staining, bFGF was clearly visible in chromaffin cell grafts. As expected, kidney cell grafts contained no bFGF. Further, the presence of bFGF in the grafts correlated with groups that showed behavioral recovery of spatial memory, as measured in the Taz-mate. These results indicate that bFGF may be involved in producing the effects of chromaffin cell grafts to cerebral cortex. (Supported by the Alzheimer Society of Canada and the Medical Research Council of Canada.)

473.3

HYPERTHALAMIC ANATOMICAL CORRELATES OF GraftING IN THE HIPPOCAMPAL FORMIC OR THE CORPUS STRIATUM. R. E. Cost and T. E. Zuccol. UCLA, Santa Monica, CA 90404.

Grafting of embryonic neural tissue into the adult brain has been shown to be a viable method for treating a variety of neurological disorders. This study examines the histological effects of grafting on the thalamus, hippocampus, and corpus striatum. Grafts were made unilaterally into the thalamus of adult rats, and then the rats were sacrificed at various intervals post-grafting. The histological effects of grafting were examined by staining the tissue with hematoxylin and eosin. The results showed that there were no significant histological differences between the control and experimental groups. However, there was a trend towards an increase in the number of neurons in the grafts compared to the control tissue. This trend was more pronounced in the thalamic grafts than in the hippocampal or corpus striatal grafts. These findings suggest that grafting may have a beneficial effect on the development of the host brain, possibly by promoting neuronal growth and survival.
473.5

The major input to the basal ganglia is the excitatory input from the cerebral cortex to the medium spiny striatal neurons. The purpose of this investigation has been to reconstruct the cortical pathway by using fetal cortical tissue transplants. Frontal cortical grafts were performed by aspiration in adult rats. After 15 days animals received grafts of 16-day gestational cortical tissue into the lesion site. Connections from the graft to the host striatum were studied after 6 weeks using immuno-staining for neurofilaments and anterograde tracing with Dil. SMI-31 and SMI-35 immunoreactive neurites were found crossing the interface with corpus callosum and striatum with a relatively high frequency (13.7 and 29.8 /mm of interface respectively, but only rarely entering the adjacent cortex. Dil tracing demonstrated a large number of graft-derived neurites projecting from 0.5 to 0.8 mm into the host caudate putamen. It is concluded that fetal cortical grafts in adult animals send limited projections into the host caudate-putamen.

473.6

Several studies demonstrate that fetal neocortical tissue will survive, grow and form axonal connections with the host brain after transplantation into newborn rats with cortical aspiration lesions. In the present study fetal neocortical grafts were transplanted into Long-Evans, black hooded rats that sustained hypoxic-ischemic brain damage at 7-8 days of age by permanent right common carotid artery occlusion under methoxyfluorane anesthesia followed by hypoxic exposure (8% oxygen) for 2-2.5 hr. Twenty-one out of 25 animals survived this procedure. One week later, 1-2 mm^{3} neocortical grafts obtained from E13 fetuses were transplanted into the right hemisphere of these animals just caudal to the coronal suture at 1-2 mm from the midline. All animals survived the transplantation procedures and were sacrificed at 2 (n=8), 4 (n=6) and 6 (n=7) weeks after transplantation. Brains were cut frozen at 30 mm and processed for acetylcholinesterase (AChE) immunocytochemistry and Nissl stain. Generalized atrophy and shrinkage of the right cerebral hemisphere was seen in 20/21 animals. Various degrees of ischemic brain lesions with neuronal loss were observed in several structures including the cerebral cortex, thalamus, hippocampus, and striatum. Well developed transplants were found adjacent to the ischemic area. AChE positive fibers were seen crossing the transplant-host interface proving evidence that the grafts became integrated into the host brain circuitry.

(Supported by NIH Grant 13230 and the Potts Foundation)

473.7

Monoaminergic neural transplants to the rat frontal neocortex have been demonstrated in our laboratory to alleviate depression in animal models. These findings suggest that neural transplants may provide a long term source of monoamines to correct the central imbalance of serotonin (5-HT) and noradrenergic (NE) functioning implicated as the cause of depression. In order to support this suggestion, grafted monoaminergic tissue must survive well and continue to produce high levels of monoamines. Using electron microscopy the ultrastructural characteristics of 5-HT-containing pineal gland tissue, NE-containing adrenal medulla tissue, grafted pineal and adrenal medulla tissue and control tissue were examined at least six weeks following transplantation. The transplanted pinealocytes maintained their characteristic in situ features of numerous and dense mitochondria, a highly convoluted nucleus and the presence of lipid droplets. Similarly, transplanted adrenal chromaffin cells retained their in situ cuboidal morphology and displayed numerous granules. Neither type of graft was highly vascularized. The grafted transplants contained collagen matrices separating the pinealocytes from the chromaffin cells. Immunohistochemical studies indicated that the surviving chromaffin cells continued to produce tyrosine hydroxylase and dopamine-β-hydroxylase and the pinealocytes continued to produce 5-HT. Sixth month old pineal implants also appeared to sprout densely into the surrounding host parenchyma. These results demonstrate that the monoaminergic tissues retain many features of their in situ morphology when transplanted to the frontal neocortex of rats.

473.8

Previous findings in our laboratory have indicated that the transplantation of monoaminergic tissue into the frontal neocortex of rats can reduce behavioral deficits in rodent depression models. If the reduction in behavioral deficits by the transplants is due to the release of neurotransmitters, it should be blocked by specific monoaminergic antagonists. We have shown that both alpha and beta adrenergic antagonists not only reversed the effects of the transplants on the behavior but in fact exacerbated the deficit, suggesting changes in receptor sensitivity. This study investigates this effect at the receptor level. Monoaminergic tissue (adrenal medulla, pineal gland, or a combination of both) was transplanted into the frontal cortices of separate saline- and scopolamine-treated animals prior to transplantation as controls in other groups. Each group was treated with imipramine (15 mg/kg/day; ip) for six weeks. Six weeks later animals were tested for behavioral deficits using the forced swimming test. Following testing, a small section (5 x 5 x 7 mm) of cortex was removed from around the transplant of each animal and the transplant was discarded. The remaining tissue underwent receptor binding studies. 8-hydroxy-2-(di-n-propylamino)tetralin was used as a ligand to investigate alpha-1 binding sites while [125I]iodocyanopindolol was used for beta binding sites. In these initial studies, K_{d} concentrations of pindolol were used to screen for any changes in binding site sensitivity or concentration. No significant differences in binding were seen between transplant and control groups for either ligand (pindolol: 3.6-4.1 fmoles/mg tissue wet wt, pindolol: 4.5-5.4 fmoles/mg tissue wet wt). However, binding was significantly reduced in imipramine treated animals. These results suggest that the previously noted behavioral and pharmacologic changes in the transplanted animals are not associated with a corresponding change in the number of monoaminergic receptor sites.

473.9
Morphological assessment of grafted cortical neurons, J. Link*, M. Wood and D. J. Clarke. Dept. of Human Anatomy, University of Oxford, South Parks Road, Oxford OX1 3QX, U.K.

The morphology of cortical neurons grafted near/or into the rat striatum was studied by means of intracarier Lucifer yellow injections in fixed slices. Rat donor cortical tissue (from postnatal day 1 old rats; AO strain) as well as mouse donor cortical tissue (prenatal E 19; C3H/EH strain) was grafted as solid pieces (a 4 mm of 4 mm of donor tissue was inserted via a Hamilton syringe) in 8-12 week old rats (AO strain). Animals were immunostained with antibody against the HNK-1 receptor. 40-60 days postoperatively animals were perfused with buffered 2% paraformaldehyde and 1% glutaraldehyde. 120 μm vibratome sections were taken at the site of the transplant. The graft could be identified by a surrounding rim of astrocytes after incubation of the slice in the DNA-stain DAPI for 10 min. Cortical neurons (over 50 neurons in each transplant) were intracarier filled with Lucifer yellow, DAB-photoreactive, embedded in resin, photographed and drawn with the aid of a camera lucida. In addition, tissue was prepared for electron microscopy to study the ultrastructural morphology and synaptic inputs of the injected neurons. In general, no cortical lamination could be observed in the grafted cortical tissue, but neurons were loosely packed throughout the graft. Two major cell types could be identified. The majority were spiny neurons (95%), of which some could be described as pyramidal-like with somata, ranging in size between 10-20 μm in diameter. The remaining 5% represented non-spine neurons with a basket-like morphology. dendrites of both cell types were never seen to cross the astrocytic border, but some main axons and axonal collateral were found to leave the graft. On the basis of light microscopic observations no difference was found between mouse and rat grafted cortical neurons. In conclusion, we have shown that grafted neurons retain some characteristic features of cortical neurons, although there appears to be a greater preponderance of spiny neurons. This may reflect an immaturity of the graft tissue or a response to the aural environment.

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THURSDAY AM

In normal animal light flashes suppress acoustic startle reflexes. In light blinded animals inhibition is lost and replaced at certain intervals by an amacrine peak of excitation, which is not seen in enucleated rats. Fischer 344 rats were blinded by exposure to continuous fluorescent light and half of the animals were gifted with dissociated fetal retinal cell into 1 eye. Compared to non-grafted controls, grafted rats showed both statistically significant reductions of amacrine facilitation and increases in inhibition (Exp: 1: del Cerro et al, Neuroreport, 1:259, 1991). To test for the specificity of this effect, grafted rats were gifted with fetal retinal cell homogenates (Exp: 2: N=20), or dissociated perinatal cerebellar cells (Exp. 3, N = 19), with control rats left untransplanted in each experiment. In contrast to the grafted rats of Exp. 1, the grafted animals in Exp. 2 and 3 were not different from their controls. Histologically, clusters of photoreceptor cells were consistently found in the retina of Exp. 1 animals, but not in those of Exp. 2 or 3. We conclude that only retinal grafts of living fetal retinal cells partially repair the blindness caused by the action of continuous light exposure.

(Supported by EY 05262, the Rochester Eye Bank, and private gifts.)

474.3 HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS OF Y79 RETINOBLASTOMAS IN VIVO. D. D. HORROTT, T. C. C. DEL CERRO, M. DEL CERRO, AND C. H. CHADLER. Department of Neurobiology, University of Rochester Medical School, Rochester, NY, 14624 and the National Institutes of Health, Bethesda, MD.

Retinoblastoma is the most common intraocular neoplasm of childhood. Continuous cell line, such as Y79, have been used primarily for analysis of tumoral cell growth and phenotype in vitro. Recently, we developed a useful cell line model in vivo model of retinoblastoma which involves subretinal transplantation of Y79 cells into immunosuppressed Fischer 344 rats (ARVO '92). Thirty to sixty days following transplantation, we examined the histopathological properties of Y79 tumor tissue. The tumors are formed by fast-dividing, pleomorphic cells which invade the host retina and vitreal cavity. These cells are immunoreactive for the neuronal marker MAP-2 among others, but remain negative for S-antigen. These tumors showed no sign of a glial cell phenotype in vitro, as the only GFP immunoreactivity present was comprised of host-derived Muller cell fibers which traversed layers of tumoral tissue. Our results show that intracocularly grafted Y79 cells survive, actively grow, and exhibit a primitive neuronal phenotype in vivo. Furthermore, these cells, even as xenografts, fully retain their features of poorly differentiated, highly invasive, neuro-tumoral cells.

(Supported by NEI 05262, T22AG01007, EY00947, generous private gifts, and the Rochester Eye Bank.)


Human immunodeficiency virus type 1 (HIV-1) infection is highly specific for its human host. To study HIV-1 infection of the human nervous system, outside of the human body, we have established a small animal model of the disease. Secondary xenografts of human fetal brain or neural retina is transplanted into the anterior chamber of the eye of adult rats immunosuppressed with Cyclosporine A. Tissue procurement was in strict accordance with established guidelines. The human xenografts vascularize, form a blood-brain-barrier, and differentiate forming neurons and glia. The xenografts can be infected with cell free HIV-1 or with HIV-1 infected human monocytes. Analysis by polymerase chain reaction (PCR) revealed HIV-1 sequences in DNA extracted from xenograft tissue exposed to HIV-1 virions, and in-situ hybridization demonstrates HIV-1 RNA localized in macrophages and multinucleated giant cells. Pathological damage was observed only in neural xenografts containing HIV-1 infected human monocytes supporting hypothesis that these cells are neurotoxic. Interestingly, the host retinal neurons were unaffected. This animal model allows the study of the direct and indirect effects of HIV-1 infection on developing human fetal neural tissues, and may be useful in the evaluation of antiviral therapies, which must target HIV-1 brain infection.

Supported by AI 32305-01, EY 09217-01, and generous private gifts.

474.5 ENHANCEMENT OF SURVIVAL OF INTRACRANIAL RETINAL GRAFTS BY ANTIGEN-SPECIFIC IMMUNE DEVIATION. Lake OJ, J. Wayne Streilein, Department of Microbiology and Immunology, University of Miami, Miami, FL, USA.

The ultimate goal of our studies is to implant functional retinal grafts into the blind eye to restore vision. In order to explore the possibility of using immunological manipulation to enhance survival of immunocompetent grafts, we implanted human retinal grafts from newborn BALb/c mice into one eye of C57BL6 mice, which induces immune deviation (ACAID). In parallel experiments, conventional immunity was induced by implanting similar grafts into the subcutaneous space. Control C57BL6 mice received a sham operation. Two weeks later, a second graft (identical to the first) was implanted into the contralateral eye of all recipients. The results revealed that all control mice rejected the test graft by 30 pd, at which time the graft size was reduced to 24% of original size. Mice which received prior SCo grafts rejected BALb/c retinal test grafts sooner. In contrast, mice which developed ACAID maintained their retinal test graft for an extended time. In this ACAID group, the graft in the first eye retained 60% of its original size by 50 pd and the second graft in the contralateral eye maintained 63% of its original size by 36 pd. Microscopic examination showed the graft in the ACAID group to have well-differentiated cell layers which resembled the structure of the normal retina. In contrast, AC retinal grafts displayed obvious histologic regression in both control mice and mice which developed a conventional immunity. These results suggest that 1) antigen-specific suppression (ACAID) can be used to enhance the survival of intracranial neural retinal allografts and 2) immunological manipulation may provide a novel way to prevent rejection of intracranial neural retinal grafts.

Supported by NEI Grants EYO9595 and EYO5678 and a grant from the Retinitis Pigmentosa Foundation.


We are examining the development of transplanted rat olfactory bulbs (OBs) using antibodies against OMP, GAP-43 and PDGFB-B. OMP identifies mature primary olfactory neurons (ONS), GAP-43 is a marker for growing axons and PDGF-B is heavily localized in the specialized glia that ensheathe the ON. Donor OBs are taken from fetuses of embryonic days 14-15 and transplanted directly into the cavity produced by removal of an OB in one day postnatal rats of the same strain. Adjacent 8 µm paraffin sections are examined using the three antibodies. Both OMP and GAP-43 are common to many large fiber bundles within the transplanted OB. Fiber bundles in sections identified with GAP-43 antibody (AB) have sharply defined borders and are slightly smaller than homologous bundles in adjacent sections reactive with OMP AB. The borders of OMP reactive fiber bundles are diffuse and may represent collateral branches around the bundle. In adjacent sections reactive with PDGF-B, photoreceptor bundles are often (but not always) surrounded or enmeshed by reactive cell bodies and fibers. Scattered PDGF-B reactivity is also seen within some fiber bundles. The relationships between fiber bundles identified with OMP, GAP-43 and PDGF-B AB remain similar at survival times ranging from 2 weeks to several months and generally support the notion that the transplants are forming a functional architecture, seen in the normal OB. Antibodies kindly provided by Dr. Frank Margolis (OMP) and Mochida Pharmaceutical Co. (PDGFB-B). (Supported by NIH Grants NS05978 and HL18405. L.E.W. is an affiliate of the CDMMC).
474.7


We are examining the axonotomized correlates involved with potential recovery of function in the rat under varying conditions (synaptic, neuronal, olfactory bulb (OB) transplant, neocortical olfactory bulb lesions (NOBL), and adult OBLs (AOBL)). The NOBL and OBL animals can eventually find hidden cookies as well as normal rats but the NOBL animals do not show recovery. Since the NOBL rat does not display general learning disabilities in passive avoidance tests, the loss of cookie-finding ability appears to be specific to olfactory ability. Histological analysis, blind of the behavioral results, was used to place the animals into groups. Techniques included: olfactory marker protein (OMP) immunocytochemistry for olfactory nerve (ON) preservation, cell and fiber staining, and triiodide autoradiography for donate-labeled transplant verification. All animals with incomplete OBLs were excluded from the study. Immunization of brain tissues by ON was achieved only in the OBL and NOBL but not in the AOBL and correlated perfectly with recovery of olfactory ability. Analysis of ON fiber reinnervation patterns in the NOBL revealed that ON targets included olfactory peduncle (ONP) and olfactory cortex (OC). Analysis of NOBL rats revealed that ON innervation of OBL was usually accompanied by innervation of OP and OC. These results suggest that in the absence of secondary OB neurons, ON axons directly with 'tertiary' neurons in the pathway and that this novel connection in the NOBL rat may be responsible for recovery of olfactory ability. It is not possible at present to say whether it is ON innervation of OBL or OPL/OC or both that is responsible for recovery of olfactory ability in the NOBL rat. (Supported by NIH Grant NS 09678; L.E.W. is an affiliate of the CDMRC.) OMP was kindly provided by Dr. F.L. Margolis.

474.8


Using lesion-degeneration methods it has previously been shown that successfully transplanted (TX) olfactory bulbs (OB) send their axons to appropriate target areas in the adult host brain (Weusten et al., 1990 Neurosci. Lett.). We are using WGA-HRP transport to study reconnnectivity in the developing TX OBs in Sprague-Dawley rats. Time-mated dams received subcutaneous injections of tritiated thymidine on embryonic days (E) 12-14. OBs from fetal rat donors of E 14-15 were immediately grafted into neonatal rats in the site from which the host OB had been removed. Following survival times of 2 weeks and longer, 0.1 μl of a 2 per cent WGA-HRP solution is injected into the TX OB and subjects are perfused after 24 hours. Alternate frozen sagittal sections are processed using TMB as the chromagen or olfactory marker protein (OMP) immunolocalization. Autoradiography will be included to verify that the injections remained within the TX OB. WGA-HRP transport is seen in fibers from the TX OB into layer I of the host anterior olfactory nucleus (AON) and piriform cortex (PC) and in cell bodies in layers II and III of the AON and PC. OMP material shows that primary fibers are seen within the TX OB and in the peduncle. These preliminary findings reaffirm that the axons from a TX OB make connections with some appropriate areas of the host brain, and also suggest that axons from cells in the target areas of the host brain innervate the TX OB. Supported by NIH Grants NS 09678 and HD 02274. LEW is a research affiliate of the CDMRC. *Ani-OMP kindly provided by Dr. Frank Margolis.

474.9

SYNAPTIC CONNECTIVITY OF SEROTONIN RAPHE TRANSPLANTS IN THE SUPRACHIASMATIC AND SUPRAOPTIC NUCLEI IN ADULT RATS. O. Britton1, 2, S. Bouquet1, M. Geffard1, 2 and A. Pradat2. 1Lab. de Neurobiologie et 2Lab. de Neurobiologie Cellulaire et de Fonctionnelle, CNRS, Marseille, and 3Lab. d’Immunologie et Pathologie, Univ. Bordeaux II, France.

We have previously reported that cell suspensions of fetal mesencephalic raphe, transplanted at mid-distance between the suprachiasmatic nucleus (SCN) and the supragnostic nucleus (SCN) in adult rats after intraventricular treatment of 5,7-dihydroxytryptamine, induced partial 5-HT reinnervation of the SCN vs hyperinnervation of the SON. We have further investigated the ultrastructural relationships of reinnervating vs normal 5-HT axon terminals in both nuclei following intranucleoduction and systematic sampling on 4-12 consecutive sections. About 48% of the 5-HT-positive terminal profiles were found in the ventral part of the normal SCN (ntv) and in the outer part of the normal SON (ntv from 4 animals), where they normally predominate, showed synaptic membrane enlargements. Graff-dense 5-HT terminals in the same proportion of the SCN (ntv, from 4 rats) revealed a higher number of varicosities (around 46%). The frequency with which 5-HT varicosities made synaptic contacts was also found to be equally high between the SON, from normal and grafted rats (more than 40%). These results indicate that the indication of reinnervation after grafting are not solely dependent on the mode of termination of normal 5-HT axons in the denervated territory. Target-specific trophic influences, i.e. target-derived stimulatory or inhibitory signals, should also be determinants in promoting graft-induced reinnervation. Such influences, together with in vitro programming of transplanted neurons committed to supply 5-HT fibers to the SCN and/or the SON, could also account for the fact that, irrespective of the extent of reinnervation (hypoc- or hyperinnervation), the new 5-HT fibers in both nuclei re-established the same relative features as in control tissue.

474.10


Transplants of the fetal hamster hypothalamic suprachiasmatic nucleus (SCN), the site of a circadian pacemaker, restore rhythmic behavior to SCN-lesioned hamsters (Lehman et al., J. Neurosci. 7:362). We explored the use of three-dimensional (3-D) extracellular matrix consisting of type I collagen (type I) as an alternative method of culturing SCN cells for use in grafting studies. (813) anterior hypothalamic cells were dissociated by a combination of gentle trituration and enzymatic treatments. Cells were rinsed and plated at 5 x 10⁶ cells/ml on 16mm wells on either the 3-D matrix or control poly-D-lysine coated coverslips. At various intervals following seeding (1, 4, 7, and 14 days) cultures were rinsed with serum-free media and fixed with 4% paraformaldehyde. The 3-D matrix was processed for immunocytochemistry (Dudley et al., Peptides 10:205) to detect a variety of neuronal and glial markers. 3-D matrices contained far greater numbers of isolated neurons and few glia in contrast to the clusters of neurons and glia seen in poly-D-lysine cultures. A 4 days positive staining for neuron-specific tubulin (class III, type II) was abundant at 14 days staining was decreased. Neurophin immunostaining suggested that SCN neurons survived in the 3-D matrix. The matrix may be useful in providing a scaffolding for neuronal cultures to be used for grafting studies, including those in which isolated SCN neurons are tested for their ability to restore rhythmicity. (Supported by NIH ROI NS28175 to M.N.L.)

474.11


Ageing disrupts multiple diurnal rhythms that are regulated by the SCN. C-Fos expression in the SCN is induced by light, and may be part of the mechanism for photic entrainment of the circadian pacemaker in the SCN. The purpose of this study was to determine whether transplantation of fetal SCN into middle-aged rats can influence light-induced C-Fos expression in the host SCN. We transplanted fetal SCN transplants into the third ventricle of middle-aged female constant estrous rats (9-12 mo). Sham operated middle-aged and young (6 mo) rats served as controls. After one month, rats were ovariectomized (dox) and treated with estradiol (day 7) to maintain a constant and similar hormone environment in both age groups. On day 11, rats were transcardially perfused within 90 min before and after lights on. Brains were prepared for immunocytochemical localization of c-Fos (Cambridge Research, OA-11-823). Young rats exhibited virtually no c-Fos expression in the liver prior to lights on, and a dramatic increase in c-Fos expression observed in young rats by 90 min. In contrast, c-Fos-containing cells are evident in some middle-aged rats prior to lights on. Transplantation of the fetal SCN into middle-aged rats appears to reinitiate the pattern of c-Fos expression observed in young rats; that is, no C-Fos expression was detectable before lights on. The results suggest a possible trophic influence of the donor SCN on the host SCN function. Supported by NIH AG00224.
475.1

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475.2

NIAGASSA FOR NEUROTOXIN-TARGET RECEPTOR BINDING BY FLUORESCENT LIQUID EXCLUSION ANALYSIS (FLEA). D. Yoshikawa

475.3

GLIAL SWELLING IN CA1 STRATUM RADIATUM IN RESPONSE TO OSMOTIC AND ELECTRICAL STIMULATION. P. Oishiho;

475.5

MATERIAL: A Fluorescent Bicarbonate Compound for Intracellular Labeling Of Neurons In Fixed Slices. W. L. Li, M.M. Babahani; and M. H. Hirt; M. T. Slepe.

475.4

AFFERENT CONNECTIONS OF NUCLEUS RAPHÉ PALLIDUS. A STUDY WITH FLUORESCENT TRACERS IN RATS. N. M. Nogueira, F. H. Beilharz, D. Dep. De Fisiol.

475.6

IMMUNOPEROXIDASE LABELING OF FLUORO-RUBY. H. T. Chang.

Supported by NIH NS20643, 24694, 29218 & DC 00437.
475.7


Lipofuscin deposition of neuronal pathways since Gendelman et al. (Development, 1987, 101:697-713). However, continued reports of cell loss (transcellular) movement of the dyes have been made by a number of laboratories since this time. The embryonic rat visual system was used to examine what conditions affect cell movement of these dyes and if cell type and cell interactions are involved.

Midgestation (E15.5) rat embryos were immersion fixed in buffered paraformaldehyde. Dil, DiA, or 4d10AASP (Molecular Probes; Eugene, OR) were prepared and applied to the optic disk. Brains were dark incubated 2-12 weeks at room temperature, sectioned biweekly, and mounted and photographed the same day. All dye labels similar cell types, but at different rates (Dil/DiA at -0.18 mm/day; 4d10AASP at ~0.21 mm/day). After incubating 2 weeks, 1) Dil, DiA, and 4d10AASP were now prominent on the optic disk. A few D10AASP and a few, D10AASP, many, midline glia at the optic chiasm, 3) neither Dil, nor DiA, label glia along the optic tracts, 4) 4d10AASP labels a large number of optic axons, ganglion and Müller cells in the opposite eye, most midline glia at the optic chiasm and glia along both optic tracts. After 8 weeks, 4d10AASP labels optic radiations into cortex, and a glial compartment dividing dorsal and ventral thalami. All dye labels more of the same cell type with interconnections.

These results suggest that, 1) the dye move cellficial via specialized contacts between axons, and axons and glia, within the optic chiasm, diencephalon, brainstem. 2) Amounts of transcellular labeling correlates with the rate of diffusion and length of incubation.

Supported by grant EY0508 (VAC), core grants EYO1662 & HD15052.

475.8

RETROGRADE LABELING OF EMBRYONIC RAT SEPTAL NEURONS FOLLOWING IN VITRO HIPPOCAMPAL INJECTIONS OF FLUORESCENT TRACERS. A. King & M.R. Hedges. University of Florida College of Medicine and V.A. Medical Center, Gainesville, FL 32610.

A technique was developed in which tracer substances were injected into isolated brains and retrogradely transported and analyzed. Specifically, septal neurons that project to the hippocampus were retrogradely labeled using FITC, fluorescent microspheres, tetramethyl rhodamine dextran days E18, E21, and P1. The embryonic rat brains were removed and immediately immersed in oxygenated Tyrode’s buffer. The brains were then incubated in a humidified chamber, bilaterally throughout the rostral-caudal extent of each hippocampus. Each injection (total of 4 hippocampi) of 10 to 30 μl DAB (dextran) was delivered through a microopette. Following 17 h of transport time in oxygenated Tyrode’s, each septal afferent was subsequently processed with DAB, and visualized in an epifluorescent microscope. The males were死刑ized in 1 ml of perfused saline and the brains were analyzed. The cultures were fixed (4%parafomaldehyde) and stored in phosphate buffer for immunocytochemical analysis. This technique will allow the identification and experimental manipulation of a subpopulation of septal cerebral septal neurons, in particular those septal neurons forming the septohippocampal pathway. Supported by the Veterans Administration, NIAAA AA00200, and A.M.H.

475.9

FLUORO-GOLD REVISITED: TOXICOCLOGICAL AND PHARMACOLOGICAL PROPERTIES. L.C. Schmued1, J.F. Bowyer1, C. Beltranino1, H.W. Birnbaumer1 & W. Slikker2, Division of Neurotoxicology, NCTR/FDA JAFCO, AR 7E209-R902 and 2Dept. of ENT, Univ. of Virginia, Charlottesville, VA 22908.

Since its introduction in 1985, Fluoro-Gold (FG, hydroxyethylaminostain) has been widely used as a retrogradely transported fluorescent tracer to reveal neuronal connectivity. However, little is known regarding its neurotoxic or pharmacological properties. To address its neurotoxic potential, FG was injected into the rat striatum at different concentrations (2.5-10%), volumes (100-250 μl), and vehicles (saline and 1 M cacodylate). Survival ranges varied from one week to one day. The tissular response was also compared to that observed in the olfactory bulb, cerebral cortex, hippocampus, amygdala, striatum, caudate, thalamus, and spinal cord. The data from a large number of these studies to identify the FG-induced neuronal and neurotoxic. Neurotoxic necrosis of neurons was identified in the cerebral cortex, brainstem, and spinal cord. FG was found to cause cell damage in these regions. The results of this study suggest that the potential of FG to cause neurotoxicity is similar to that of other fluorescent tracers.

475.10

LABELING OF SPECIFIC CELL TYPES IN THE RAT CNS BY INTRAVENTRICULAR INJECTIONS OF BIODIOTIN. A.J. McDonal1 & E. Mascagni. Dept. of Cell Biology and Neurosciences, School of Medicine, University of South Carolina Sch. of Med. Columbia, SC 29208.

Recent experiments (Mascagni et al., 1989) have demonstrated that large injections of biotin into the lateral ventricle (or brain) produce labeling of particular neuronal subpopulations in the rat CNS. Rate were perfused 24 hours following injection and stained with the ABC technique. In many cases the staining of neurons was very complete and resembled that obtained with the Golgi technique. Regions containing labeled cells included the olfactory bulb, cerebral cortex, hippocampus, amygdala, striatum, and cerebral cortex, and dorso horn of the spinal cord. Only particular cell types were labeled, while all nonpyramidal neurons were strongly labeled in the cerebral cortex (FG) or neurons in the ventral horn (BDA). Labeled cells in specific brain regions are small local circuit neurons. Several of these cells show short survival times (4-6 h) exhibited strong labeling of glial cells and light labeling of a large percentage of neurons in many brain regions. Cells with 6-8 survival times exhibited no labeled cells. Injections of d-biotin did not produce neuronal labeling. Injections of biotinamide (neurobiotin, Vector Labs) produced a pattern of labeling that was much different from that produced by biotin. The results of this study suggest that there is differential uptake and retention of biotin by populations of CNS neurons. This study system may be associated with biotin-dependent metabolic pathways in these cells. Double-labeling studies are in progress to further characterize neurons that exhibit biotin uptake. Supported by NIH Grant NS27333.

475.11


A bioimolecular recording and immunocytochemical labeling were used to determine the ultrastructure and synaptic association of physiologically characterized pyramidal neurons in the superficial layers of the rat frontal cortex. After intracellularly, individual neurons were filled with biocytin, and the animals were transcardially perfused with acrolein. Sagittal serially collected sections were processed for visualization of biocytin using avidin biotin peroxidase. Sections containing the filled neuron were processed using immunogold silver for detection of GABA or tyrosine hydroxylase (TH), and a marker of catecholaminergic afferents. Filled neurons had branched and spiny dendrites as seen by light microscopy. At the ultrastructural level, filled perikarya and dendrites had irregular (ruffled) contours. Although these were densely filled with peroxidase, synaptic contacts were usually identifiable. In dually labeled sections, i) GABA immureactivity was seen in perikarya, dendrites and terminals, some of which formed synapses on proximal filled dendrites, and ii) GABA axons contacted both filled and unlabelled dendrites in the superficial layer. The combined use of in vivo intracellular labeling with ultrastuctural identification of transmitter provides an important method for demonstrating the synaptic basis for modulation of cortical activity. (Supported by grants MH30078, DA04600, HL10974).

475.12

COMBINED ANALYSIS OF CALCIUM BINDING PROTEINS, GAMMA AMINOBUTYRIC ACID, TRANSGREDUCTION OF WHEATGERRM AGGLUTININ HORSERADISH PEROXOIDE AND N-TERGREDUCTION IN THE SPUTUM OF THE PRIMATE. Amanda M. Miller, Tracey, Doh Rahab and Henry J. Baldwin, III Department of Anatomy and the W.M. Keck Foundation Center for Integrative Neurosciences, University of California, San Francisco, California, 94143.

Our laboratory has used the exploratory search of several neuroanatomical techniques simultaneously to analyze projections in the primate central nervous system to neuronal populations that have been characterized for the presence of neurotransmitters and calcium binding proteins. As an example, one is factinhibits, as part of a series of studies in which our laboratory is currently involved, it is controlled with wheatgerm agglutinin-horsedant peroxidase (WGA-HRP) in somatoaxoeritory/motor cortices for antergrade transport in cortico- spinal/halamic/reticular pathways and cell bodies and axons in motor cortex in the primate, area 4, 4A, area 2, cortico spinal/spinal afferents. According to the protocol described above, GABA and biotinamide were injected into the thalamus with 2% paraformaldehyde and 2% glutaraldehyde. pH 7.4 at room temperature. The brains were then fixed in 4% formaldehyde in 0.1 M phosphate buffered saline. Vibratome sections of 100μm were reacted with immobilized biotinylated (for GABA) or paraformalin coated with avidin-biotin-cytochrome c (for biotinamide) and stained with immunohistochemistry the same day. This research was funded by the National Institute of Health (NS 2172).

Our technique allowed the identification of neurons that project to the hippocampus and the identification of neurons that project to the thalamus. These results suggest that the development of a neuron-specific probe for neuronal markers may have important implications for the study of neuronal circuits in the primate.
475.13
LIGHT AND ELECTRON MICROSCOPIC STUDIES OF BOUTONS ON ISOLATED RAT NEURONS. W.L. * and M.D. DeSantis. Dept. of Biological Sciences and WAMI Medical Program, University of Idaho, Moscow, ID 83843, USA
The size was measured on isolated neuronal somas using scanning electron microscopy (SEM). Neuronal cell bodies were isolated from the trigeminal motor nucleus by enzymatic digestion of the peripheral nerve tissue, and the isolated somal neurons were identified by electron microscopy and then examined by SEM. They ranged in size from 23.0 to 41.6 um average diameter (n=23). Preparation for SEM resulted in an average shrinkage of the soma by 20% of its diameter. There was a direct relationship between the size of cell body and the amount of shrinkage. SEM revealed bulbous structures from 0.6 to 3.6 um in size at the surface of neuronal somas. Our interpretation that they were boutons was confirmed when transmission electron microscopy (TEM) was used to study the same somas after SEM. The surface of the isolated somas was generally free of adhering elements except for the boutons. (Supported by Idaho State Board of Education grant 92-010).

475.14
GJAP (gial hyaluronic acid binding protein) is a glycoprotein which binds to hyaluronic acid in the CNS. Functionally, GJAP is thought to inhibit neurite adhesion and outgrowth. In this study, the ultrastuctural localization of GJAP was performed in GA-fixed spinal cord and cerebellum, the two portions of the CNS with the highest GJAP staining. The localization was performed via an indirect immunoperoxidase staining with the monoclonal antibody 2C3 as the primary antibody. Animals were anesthetized and then perfused via a gravity flow system through the left ventricle, first with 50C of PBS (phosphate buffered saline) pH 7.3-7.4, containing 2% procaine-HCl and then with PBS (100-150 ml). This was followed by fixation with 100-300C of PBS at pH 7.3-7.4 containing 2.0% pararaffinoldehyde, 2.5% glacial acetic acid and 1% CPC (cycloptiuturdefinium chloride); the CPC serves to stabilize the water soluble hyaluronic acid in the extracellular matrix to which the GJAP binds. After perfusion, the brain and spinal cord were removed and stored overnight (12-16 hrs.) in the same fixative. Vibratome sections were subsequently collected and stained by indirect immunoperoxidase using the DAB protocol of Streit and Reubi (1977). Electron micrographs will be presented to illustrate the labelling of GJAP in both the spinal cord and the cerebellum. This research supported in part by NIH Grant NS 13034 and V.A. Gen. Res. Service Merit 0022.

475.15
A PRE-EMBEDDING TRIPLE-LABEL EM IMMUNOHISTOCHEMICAL METHOD AS APPLIED TO THE STUDY OF MULTIPLE INPUTS TO DEFINED THE SUBSTANTIA NIGRA NEURONS. A. Raineteau, K.D. Anderson and E.J. Karle. Dept. of Anat. & Neurobiol., Univ. of Tennessee, Memphis, TN 38163, USA
Both dopaminergic and nondopaminergic neurons are present in the substantia nigra, and the substantia nigra receives input from both enkephalinergic (ENK+) striatal neurons and substance P-containing (SP+) striatal neurons. To determine the types of nigral neurons receiving inputs and to determine whether such individual neurons receive inputs from both types of terms, we have developed an approach for carrying out pre-embedding triple-label EM immunohistochecmy, using three distinct markers.
Following fixation with a paraformaldehyde-glutaraldehyde-acrolein fixative, vibratome sections were sequentially labeled for the localization of: 1) SP + terminals using a PAP/DAB procedure with rat anti-SP; 2) ENK + terminals using a 1nm gold-conjugated secondary with mouse anti-ENK; and 3) tyrosine hydroxylase-containing (TH+) neurons using a PAP/BDHC procedure with rabbit anti-TH. The sections were then osmicated and the immunogold labeling was silver intensified. Thin sections examined with EM showed that all three labels could be distinguished at the EM level. Although individual SP + and ENK + terminals contained both TH + and non-TH + perikarya and dendrites, profiles receiving simultaneous contact from both SP + and ENK + terminals were typically non-TH+. These results show that pre-embedding triple-label EM immunohistochemistry is feasible, and that SP- and ENK- striatal terminals may be segregated on nigral dopaminergic neurons (i.e. on different neurons or different parts of the same neurons). Supported by NS 19620 & NS 28721 (A.J.)

475.16
The Na+/Ca exchanger (NCX) is prevalent in neurons and appears to be concentrated at nerve terminals; the exchanger is also present in glia (Ann. N.Y. Acad. Sci. 639:254-274, 1991). Polyclonal antisera specific to canine cardiac NCX were used to localize the NCX in adult rat hippocampus. We compared the distribution of the NCX immunoreactivity to the distribution of synaptic 1 and 2 and synaptophysis (synaptic vesicle markers), and glial fibrillary acidic protein (GFAP; a glial marker). Immunostaining of 25-mm cryosections of hippocampus was performed by an indirect immunoperoxidase procedure. The anti-NCX antibodies labeled the strata oriens and radiatum most intensely, followed by stratum lacunosum-molecular and the inner third of the dentate molecular layer where axons from both the ipsilateral and contralateral hilus terminate. The regions of the hippocampus where the neuronal cell bodies are located (the stratum pyramidale and the dentate granular layer) were poorly labeled by the anti-NCX antisera. No specific labeling in any region of hippocampus was observed with preimmune serum. Immunostaining patterns for synaptic 1 and 2 were similar to the NCX patterns although antibodies to synaptic 1 and 3 and synaptophysis, but not NCX, strongly stained the entire molecular layer of the dentate gyrus. The GFAP immunostaining pattern differed markedly from the NCX pattern. These results suggest that many of the NCX immunoreactivity is associated with synapses since synaptic 1 and 2 are present in all nerve terminals. We concluded that the NCX is present in relatively high concentration in the hippocampus, and that it is especially prevalent in some synaptic fields where it may play an important role in Ca homeostasis.

475.17
Cytochrome oxidase (C.O.) contains 13 subunits which are individually encoded by nuclear genes. Nuclear-derived mitochondrial proteins are cytoplastically synthesized as precursors carrying cleavable polypeptides (presequence) at the N-terminus, which signal the targeting of precursors to the mitochondria. In order to determine if nuclear-derived precursors are localized mainly in neuronal cell bodies or in dendrites, we generated polycyclonal antibodies against the presequence of the rat C.O. IV precursor. Immunoreactivity could be detected in all brain regions examined (cerebellum, hippocampus, brain stem, somatosensory barrel cortex, and olfactory bulb). There is a distinct heterogeneous immunohistochecmy pattern among different brain regions, laminae, and cell types. The pattern of immunoreactivity for the precursor matched that for the mature enzyme and the pattern shown by C.O. histochemistry. Antiseria were localized in both somata and dendrites in the hippocampus, where somatic and dendritic layers are spatially segregated. This is the first evidence that precursors of the rat C.O. subunit IV are detectable in the mammalian brain. (Supported by NIH grant NS 18122 to MWR)

475.18
QUANTITATIVE DIFFERENCES IN CALCITONIN GENE-RELATED PEPTIDE (CGRP) IMMUNOREACTIVITY IN RAT SPINAL CORD NEURONOPHARES IN SITU AND SLOW MUSCLES. J. Calcedo, A. Zorrilla, E. Herronboch, V. Yuste, J.X. Comellas and J.E. Escudero, Unidad de Reperc Neuro muscular, Department of Ciencias Medicas Basicas, Facultad de Medicina de Lleida, Universitat de Barcelona, E-25006, Lleida, Spain.
When CGRP is detected by immunohistochemistry it is a substantial variation on the intensity of the reaction is found among the spinal cord motoneuron population. The significance of such heterogeneity is unknown. We have explored whether or not motoneurons innervating fast or slow muscles showed correlative differential content in CGRP. Motoneurons innervating the slow soleus or the fast extensor digitorum longus (EDL) muscles from adult Sprague-Dawley rats were labeled by immunocytochemical injection of fluorescein-conjugated wheat germ agglutinin or cholera toxin (B subunit). CGRP-like immunoreactivity (CGRP-LI) was evidenced by immunofluorescence using rhodamine as a label. The intensity of CGRP reaction was measured in traced neurons with a microspectrophotometer. Recorded data from each set of samples were analyzed by means of an automated classification procedure for individual intensities in each neuronal population. The classification algorithm is based on a pattern recognition method with no initial membership defined for each individual cell. In this way an inverse frequency distribution of the intensities of CGRPLI was consistently observed when histograms from soleus and EDL-innervating motoneurons were compared: the strong CGRPLI was more frequent in the body of soleus-innervating motoneurons. When the same evaluations were done in male rats, the above mentioned clear-cut differences were not consistently observed. It is concluded that the type of motor units and/or metabolic state of the muscle fibers may influence the neuronal CGRP.
475.19

A SILVER-REDUCING AXONAL PROTEIN IN THE CENTRAL NERVOUS SYSTEM OF ADULT RATS. C.J. Tandler, A. Pellegrino de Iraldi* Instituto de Biologia Celular, Facultad de Medicina, UBA, Paraguay 2144, C1131, Buenos Aires, República Argentina. A selective 'argentaffin' staining of nerve fibers after mercuric-acetate post-fixation was reported (20th Am. Meet Soc Neurosci, Abstract 28A, 1990). The technique (Hg-Ag) also stained specifically proteins within lateral components of triads and diads in striated muscle cells (His- tochemistry 92: 271, 1989). The axonal argentaffinity is dependent on both glutaraldehyde and mercuric ions. It is not suppressed after extraction of lipids, heating in 1N perchloric acid or incubation with alkaline phosphatase. Methylation of glutaraldehyde fixed tissues abolished the Hg-Ag reactivity indicating that carbohydrate groups in protein are involved in the formation of the organic mercuri- nal responsible for silver staining. The electron micro- scope shows that the silver-reducing protein localizes in- side the axon. The procedure stained white and gray matter fibers in cerebrum, cerebellum and spinal cord but not the neuronal perikarya or their dendrites and proximal axons. In the cerebellum the basket cell axons were strongly re- active but not the parallel fibers. The possibility of the method to distinguish between functional stages of pro- teins in relation to cytotrophic variations in cytoskeleton structures is suggested.

Work supported by grants from the CONICET and UBACyT, República Argentina.

475.21

CEREBRAL ARBROPHY AND HYDROCEPHALUS IN CONTROL AND GANGLIONECTOMIZED SPONTANEOUSLY HYPTERTENSIVE RATS. D. Livingstone, P. Bann, L. Hel, F. Brain, •., W. Brain, S. Brain, F. Brain, N. Brain, and J. Brain. Stoney Brook, NY 11794-8122. The spontaneously hypertensive rat (SHR) is hypertensive, hyperactive, and hydrocephalic relative to Wistar-Kyoto (WKY), in young adults, glucose metabolism is lower in SHR than WKY and is elevated in one superior cerebellar ganglion in mesencephalic tissue 4 weeks of age. In view of these cerebral clinical features, tissue volumes and neuronal density were measured in control, ganglionectomized (Gx), and sham-operated (sham) SHR and WKY. In control SHR the volume of the whole brain and of some areas was 1.10% less than control WKY; ventricular (CSF) volume was 0.2 times larger in SHR than WKY. Neuronal density was similar in control SHR and WKY. Relative to controls, the volumes of brain and all brain areas were 15-25% less in uGx and sham SHR as well as uGx and sham WKY. In both SHR and WKY, neuronal density in some areas tended to be less in uGx and sham rats. The uGx procedure at 4 weeks of age, but not ganglionectomy per se, seems to affect brain structure and function.

475.20

ANATOMICAL ORGANIZATION OF INTRINSIC NEURONS OF THE RABBIT TRACHEA. R. Hendriks, S.R. Koopman, and D.I. Kreulen, Department of Pharmacology, College of Medicine, University of Arizona, Tucson, Arizona 85724.

The mammalian trachea is likely to be under the effenter influence of both intrinsic and extrinsic neurons. Using a variety of neuronal markers, we have investigated the anatomy of intrinsic tracheal neurons of the rabbit. Individual trachea were variously labeled with Neutral Red (Sigma, USA) and antibodies to Neuron Specific Enolase (NSE) or S-100 protein and were prepared for viewing either as wholemounts or tissue sections. All three major classes of intrinsic neurons of the rabbit trachea. The nerve cells were found to be organized into small ganglia containing less than ten cell bodies each. The ganglia were primarily between the cartilaginous rings and at the esophageal border. Most were found to lie at the surface of the tissue although occasionally ganglia could be found lying deep within the trachea. No clear pleurs arrangements for these neurons were discernible. Individual fibers of some neurons could occasionally be followed to the membrane. In conclusion, the intrinsic neurons of the trachea form a diffuse neural network that, accordingly, is likely to have a unique role in the functioning of this tissue. Support HL-46471.

475.22

PHOSPHOTYROSINE-CONTAINING PROTEINS IN THE RAT BRAINSTEM AND SPINAL CORD. J.W. Ungethr, W. Lange and J.W. Livingstone*. Department of Anatomy, University of Munich, FRG, and 1Department of Endocrinology, University of Rochester, Rochester, NY 14622, USA. The regulation of cell activity by a number of growth factor receptors and proto- oncogene products involves tyrosine kinase (TK) activity. The presence of phosphotyrosine-proteins (PY) is an index of the activity of TKs. Light- and electronmicroscopy demonstrate PY immunoreactivity in numerous nuclei of the brainstem and spinal cord of the adult rat, including central and efferent nuclei, e.g., nucleus of the solitary tract, hypoglossal, lateral reti- naeum nucleus, area postrema. Ultrastructural analysis revealed PY in the cytoplasm, in the perinuclear Golgi complex and at pre- and post- synaptic sites. Several receptor and cytosolic TKs may contribute to the PY-substrates, i.e., high overlap was evident with the presence of insulin receptors. Our results suggest that protein tyrosine phosphorylation may play an important role for the regulation of neuronal function in the adult central nervous system. (Supported by the Walter-Schulz-Stiftung, WZH, and NATO Collaborative Research Grant).

477.1

PERMEABILITY COEFFICIENT-SURFACE AREA PRODUCT OF THE BLOOD NERVE AND BLOOD BRAIN BARRIERS FOR NGF AND ALBUMIN. J.P. Podvalnik, G.L. Stone and C.J. Bart, Departments of Neurobiology and Laboratory, Departments of Neurology and Biochemistry/Molecular Biology, Mayo Clinic and Mayo Foundation, Rochester, MN 55905 USA.

Neurotrophic factors will likely be of importance in the treatment of neurodegenerative diseases of the peripheral and central nervous system. Although their administration to patients with Alzheimer's and other neurodegenerative diseases may represent a new approach to treatment of these degenerative diseases, the development of a reliable, non-invasive, delivery system is clearly needed prior to the initiation of clinical trials. We have used the in-vitro column technique to measure the vesicular blood-brain barrier for NGF. The blood-brain barrier to NGF across the blood nerve barrier was 1.726 ± 0.394 x 10^-14 ml/mg/sec (I ± S.D). When compared to the PS for normal albumin (0.101 ± 0.008), a 7-fold increase was observed for NGF. Although there is a 9-fold difference between NGF and albumin, the PS for NGF was significantly higher suggesting a possible different mechanism of uptake into the nerve. Values obtained for different brain regions ranged from 2.7±3.2 fold higher for NGF compared to albumin. No changes were observed in the residual plasma volume for either the endoneurium or the different brain regions. Data will also be presented which suggest that selective modifications of NGF can enhance its permeability into the nervous system while still preserving biological activity of the protein. Such approaches might be useful in formulating a reliable delivery system of this and other neurotrophic factors for the potential treatment of Alzheimer's and other neurodegenerative diseases.

477.2


The effects of tumor necrosis factor-alpha (TNFalpha) on the development of the CNS raise the possibility that circulating TNFalpha might enter the brain. We examined the blood-to-brain transport of recombinant murine TNFalpha across the blood-brain barrier (BBB) in 3 day old rat pups. H3-TNFalpha and [125I]-TNFalpha were injected IV, and the unidirectional influx constant (ki) into the brain was calculated from the levels of radioactivity in the brain and blood. Intraperitoneal injection of human albumin was detected for the 30 min period tested, while the Ki for H3-TNFalpha was 8.1±10-4 ml/min/g and the Ki for [125I]-TNFalpha was 1.66±10-4 ml/min/g in newborn mice. The Ki for TNFalpha decreased the Ki to 0.79±10-1 ml/min/g, which represented an inhibition of over 90% of [*]-TNFalpha entry. The Ki for [125I]-TNFalpha was 1.1±10-5 ml/min/g. The Ki for TNFalpha, however, did not alter the brain/blood ratio of [*]-albumin (11.7±2.9 ml/g brain for controls vs 11.5±2.9 ml/g for excess TNFalpha). These results show that TNFalpha does not disrupt the neonatal BBB and support the existence of a blood-to-brain saturable transport system for TNFalpha in the neonatal rat.

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476.3


Studies in animal models with high BBB (MAO-B) activity is central microvessels have suggested that circulating dopamine (DA) is present from entering brain parenchyma by the enzymatic blood-brain barrier (BBB). In the present study, blood-brain DA transport is examined in an animal model with low BBB MAO-B activity - the guinea pig. 

Cavia porcellus which is this respect is similar to humans. A vascular brain perfusion method [J.Neurochem. (1986) 46: 1444-1451] and a capacitative technique [J.Neurochem. (1990) 54: 1802-1882] were used to study BBB transport and/or binding of plasma derived DA. The initial rapid uptake (V) and rate of entry (K) of [3H]-DA into the brain tissue were both found to be almost 10 times higher that that of simultaneously infused [3H] - succrose, a metabolically inert cerebrovascular space marker. A substantial in situ DA binding to cerebral microvessels was also demonstrated. 

Brain uptake of [3H]-DA (2 mM) was significantly inhibited in the presence of 500 mM unlabeled DA (by 49%); concentrations of 1-250 mM produced further inhibition (90-100%). 

The D2-receptor antagonist apomorphine (25 mM) virtually abolished [3H]-DA uptake as evidenced by both brain homogenate and capacitative-depleted tissue. In situ DA binding to cerebral microvessels was also demonstrated. 

Brain uptake of [3H]-DA was decreased in animals treated with the MAO inhibitor pargyline (50 mM). These results suggest that DA is indeed transported across the BBB in guinea pigs by a specific MAO-independent mechanism. 

Brain uptake of circulating dopamine may be mediated by a capillary membrane D2-receptor. (Supported by Children's Hospital of Los Angeles 1102/896).

476.4


Tyr-MIF-1, a peptide with opiate-modulating properties, and Met-enkephalin, are removed from the brain by a peptide transport system (PTS-1). Since morphine given during the neonatal period is known to alter opiate function, the examination whether PTS-1 was also affected. 

Neonatal rats were injected subcutaneously (SC) during the first week of life with either morphine sulfate (MS), 

MS+naltrexone (MS+Nal), MS+methyl naltrexone (MS+Mnal), or vehicle at a dose for each drug of 50 µg/rat. On postnatal day 22, the brain-to-blood transport rate of 131I-Tyr-MIF-1 was measured as previously described (Methods in Enzymology 168: 652-660, 1989). 

Pups treated with MS had significantly smaller brain weights than controls (p<.05) or MS+Mnal treated pups (p<.001). After treatment, the transport of 131I-MIF-1 by PTS-1 in MS treated pups was over 218 faster than in control rats (p<.01) and 164 faster than in MS+Mnal treated pups (p<.05). 

These results indicate that treatment with opiates during the neonatal period can affect the development of PTS-1.
476.9
AMINO ACID TRANSPORT INTO MEMBRANE VESICLES IS NOT RELATED FROM THE BLOOD-BRAIN BARRIER.
Luminal and abluminal membranes from bovine brain endothelial cells were separated on Ficoll gradients. The membranes formed sealed vesicles that were adequate for transport studies. Transport of D-N-methylamino-l-phenylalanine was studied in both membrane populations. Luminal vesicles showed a high affinity transport system for L-phenylalanine that was independent of Na+ and H+ gradients and inhibited by other large neutral amino acids, including D-phenylalanine; it was not inhibited by MeAIB. In contrast, abluminal vesicles contained a Na+-dependent phenylalanine transport system that was inhibited by MeAIB. Similarly, uptake of radiolabeled MeAIB was stimulated by a Na+ gradient in abluminal vesicles, whereas no significant effect of a Na+ gradient was observed in luminal vesicles. The results provide direct evidence for the presence of the Na+ dependent A-system exclusively in the abluminal membranes of the BBB. These data are consistent with the location of an L-type system in both membranes.

476.11
IN VIVO LABELING OF BRAIN MICROVASCULAR TRANSFERRIN RECEPTOR USING COLLOIDAL GOLD-ANTITRANSFERRIN RECEPTOR ANTIBODY-COMPLEXES. I. Yang, U. Bickel, T. Yoshikawa, H. Weiner*, and W.M. Pardridge. Departments of Medicine and Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.
The transferrin receptor (TFR) is highly expressed at the plasma membrane of brain microvascular endothelial cells, which form the blood-brain barrier (BBB) in vivo. A monoclonal antibody to the rat TFR, OX26, has recently been evaluated as a model of brain transport vectors for drugs unable to pass through the BBB. Transcytosis of OX26 and OX26-drug conjugates could be demonstrated in pharmacokinetic studies, and OX26 was successfully used for brain delivery of a vasoactive intestinal peptide analog, resulting in a pharmacological response (increased cerebral blood flow).
To obtain ultrastructural information about the in vivo transcellular trafficking of OX26, at the abluminal side of a brain capillary in vivo, a direct receptor labeling study with 5 nm colloidal gold/OX26 complexes was performed. The complex was infused for 10 min into anesthetized Sprague-Dawley rats via the internal carotid artery (about 200 μg OX26/rat). Immediately after the end of the infusion, the animals were perfusion fixed via the ascending aorta with 2% glutaraldehyde. Vibratome and semithin sections of the brain were silver processed for transmission electron microscopy. A pattern of gold particles in defined cytoplasmic compartments resembling endosomes was seen. In conclusion, this study presents a novel approach to in vivo receptor labeling and ultrastructural analysis.

476.10
The potential for receptor-mediated transcytosis of ferro-transferrin (ITRF) and antibody against its receptor through BBB endothelia was investigated in Sprague Dawley rats. ITRF, a blood-borne peptide, conveys iron to tissues and cells. BBB endothelia exhibit the receptor for ITRF on the luminal surface. Rats were injected into the carotid artery with ITRF conjugated to HRP (60g/0.5ml; 1-3hrs) or intravenously with native HRP (300g/0.5ml, 1hr). HRP-conjugated or non-conjugated antibody to the ITRF receptor (OX26, 200-700μg/0.5ml, 5mins-6hrs) and against the Major Histocompatibility Complex Class I (OX27; 210μg/0.5ml 6hrs) was employed as controls. Non-conjugated antibodies were identified immunohistochemically. On-HRP, each of the antibodies, and native HRP labeled BBB endothelia, perivascular phagocytes and pial surface macrophages. ITRF-HRP and OX26-HRP were seen ultrastructurally within perivascular clefts; ITRF-HRP only was observed in cells of the neuropil. OX26-HRP only was localized in secretes of the Golgi complex in BBB endothelia. Native HRP and OX27 labeling of perivascula and pial surface phagocytes occurs through extracellular routes circumventing the BBB. Potential transcytosis of blood-borne ITRF-HRP and OX26-HRP through the BBB very likely occurs, but the exact intracellular pathways are elusive. Supported by NIH grant #NS18030.
**477.3** ANALYSIS OF PRIMARY AFFERENT INPUT TO RAT DORSAL HORN S. Jeffinija*, L. Kojic, T.-H. Chen and L. Urban 1. Dept. Vet. Anatomy and Neurosciences, Texas A&M University, College Station, TX 77843; 2. Dept. of Veterinary Pathobiology and Microbiology, Texas A&M University, College Station, TX 77843; 3. Dept. of Veterinary Pathobiology and Microbiology, Texas A&M University, College Station, TX 77843.

We have used the sucrose gap technique applied on the sympathetic chain to measure membrane potential of preganglionic terminals of bullfrog sympathetic ganglia (X segment). A switching conductance.

**477.4** PURIFICATION OF β-LEPTINOTARSIN-h BY IMMUNOAFFINITY CHROMATOGRAPHY R. D. Gelfinger*, J. D. Stagg, J. D. B. A. Landers, Y. L. T. Hsiu-hsia, and S. H. W. Gelfinger, Biophysics and Immunology, Department of Marine Biology and Marine Biotechnology, Scripps Institution of Oceanography, La Jolla, CA 92037, USA. 

We immunized Balb/C mice with the heretofore most purified preparation of β-LPT-h. Monoclonal antibodies were generated by cell fusion with SP2/0 parental myeloma cells. Antibodies of interest were located by determining the ability of antibody + protein G-Sepharose to remove 125I uptake activity from solutions containing β-LPT-h. We found four activity-binding antibodies, one of which (2C3-1-1) we choose for further study. 2C3-1 bound to only 8% of the activity in the preparation used for immunization, revealing the presence of at least two immunologically distinct Ca+ uptake activities. Column chromatography of β-LPT-h with 2C3-1 coupled to Affi-Gel 10 and subsequent SDS-PAGE revealed two bands of 55 kD and 60 kD.

**477.5** THE EFFECT OF CALCIUM AND SODIUM ON THE EXTRACTIVE CONCENTRATION, RECOVERY OF Dopamine BY USING QUANTITATIVE MICRODIALYSIS. A.D. Smith* and J.B. Justice, Jr., Department of Chemistry, Emory University, Atlanta, Georgia, 30322.

The point of no net flux was used to study the effects of varying the sodium (Na+) and calcium (Ca++) concentrations (conc.) on afferent nerve activity (29±2.2 mV, mean ±SEM, n=32) and blocked AP. Simultaneous intracellular recording from superficial DH neurons revealed that in cells receiving input from both small and large fibers, excitatory postsynaptic potentials (EPSPs) evoked by electrical stimulation of small myelinated afferents (0.2-10μM) to the DRG blocked electrically-evoked APs in large DRG neurons and consequently EPSPs in DH neurons. Depolarization of high potassium on DRG neurons and excitatory effect on DH neurons was not blocked by TTX. During this potassium-induced increased activity in DH neurons, EPSPs evoked by stimulation of small unmyelinated fibers were potentiated. Our data suggest that in large DRG neurons with TTX-sensitive Na channels high K evoked depolarization inactivates the Na current and blocks AP generation. As TTX-resistant Na channels require higher levels of depolarization C-fibers remain active in the presence of high K and maintain synaptic input to the DH. This difference could be responsible for the selective activation of C-afferent fibers by high K which produced EPSPs in the spinal cord. This hypothesis is further supported by the finding that when Na ions in the DRG bathing media were totally removed in the presence of TTX, both high K and electrical activation of afferents were without effect on DH neuron. Work was supported by NIH Grant NS27751.

**477.6** ELECTRICAL STIMULATION REPROGRAMS THE METABOLIC AND SYNAPTIC PHENOTYPES OF CRAYFISH MOTONEURONS. T.H. Hsiu-hsia, J.D. Gelfinger, Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto, Ontario, MS8 1A8. 

Can impulse activity in motoneurons determine metabolic efficiency? Using Rhodamine-123 (Rb) as a supravital, mitochondria-specific fluorochrome, we compared metabolic activity and synaptic physiology of crayfish tonic and phasic abdominal motoneurons. Tonic flexor motoneurons show a highly activity-dependent resistance to synaptic depression than phasic extensor motoneurons. Axonal mitochondria from the former showed significantly higher mean Rh fluorescence intensities than did the phasic axons' mitochondria (as measured using confocal microscopy). Mitochondrial metabolic inhibitors (dinitrophenol, azide, CCCP) induced synaptic depression in tonic axons and reversibly abolished the Rh fluorescence of their mitochondria, suggesting that Rb fluorescence signals metabolic competence. In vivo stimulation of an identified phasic motoneuron significantly increased both neuromuscular synaptic stamina and mean axonal mitochondrial Rh fluorescence. Axotomy prior to conditioning stimulation abolished both changes. Thus, there exists a direct correlation between mitochondrial metabolic capacity and synaptic stamina in single living motor axons, while imposed electrical activity reshapes both metabolic and synaptic performance.

**477.7** ELECTROGENIC Na-K PUMP IN SYMPATHETIC PRE-GANGLIONIC FIBRES IN BULLFROG LUMBAR SYMPATHETIC CHAIN. T. Hashisochil*, M. Hashisochil, T. Tosa*, and A.-L. Padasio, Department of Physiology, Tokyo Medical College, Tokyo, Japan and Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Oc, Canada.

Excitability of nerve terminals in vertebrate nervous system is difficult to study in a direct way. We have used the sucrose gap technique applied on the sympathetic chain to measure membrane potential of preganglionic terminals of bullfrog sympathetic ganglia (X segment). A switching amplifier (Land & Horowicz, Physiological Action Potential Recorder, Pharmacia-Wellcome) was used for simultaneous current injection and potential measurement. Injection of a current pulse (200-400 ms2) was followed by biphasic hyperpolarization of the SM stimulus electrode. A depolarization phase reached threshold for action potentials generation. HAP was unaffected by t-tubocurarine (0.1 μM) or atropine (1 μM), and thus not likely result of endogenous acetylcholine release. Removal of extracellular sodium (choline replacement), but not of Ca2+ or Cl, abolished the HAP. Na,M,K-pump blockers (3-10 μM ouabain, Na-free lithium Ringer) as well as Na-axide blocked the HAP generation; however, 2.4-DNP (0.1 μM) only depressed it. Addition of cesium (2-5 mM) caused a conc dependent increase in HAP amplitude (> 25%; 110% at ED50, 7 m Ms Cs and duration and slowing of the rate of repolarization 0.031/sec ± 0.004A, n = 4), more than could be explained by a depression of inward rectification. HAP was not accompanied by changes in membrane conductance.

These results suggest that HAP in preganglionic terminals results from activation of an electrogenic pump. (This work was supported in part by MRC and CRC)

**477.8** ALTERNATIVE SOLUTIONS OF ANIMAL IMPULSE PROPAGATION INTO A LARGER-DIAMETER REGION. R.D. Gelfinger*, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH 45435. 

Impulse propagation was simulated for unmyelinated axons whose diameter changed from 1 to 2-10 μm. The 1-dimensional cable equation was solved with a finite-difference discretization (1). The spatial integration step (Ax=3 μm) was constant, hence, the electronic step Ax/A = 0.5 was geometry-dependent (2). The Hodgkin-Huxley equations (3) defined excitability, first-order differential (4) and second-order (5) Euler integrations over very small time steps (e.g., = 0.1 μs) were kept constant during each run (6). No predictor/corrector methods were used and the electrotonic circuit was not closed. The electrotonic step was 10°C. It was assumed that Na, K, and leak maximal conductances and other properties remained constant during the increase in diameter. CV(x) at the diameter expansion if the electrotonic step in the expanded region equalled that of the smaller-diameter region. Correcting the CV(x) at the diameter expansion, CV(x) changed axially: first increased, then decreased, and finally increased at the expansion to a maximum. Impulse propagation was still possible in a single living motor axon, while imposed electrical activity reshapes both metabolic and synaptic performance.

477.1
MODULATION OF LOCAL ANESTHETIC BLOCKADE IN SINGLE FIBERS BY ACTIVATION OF NEIGHBORS IN PERIPHERAL NERVE. S.A. Raymond*, S.C. Steffen, J.G. Thalhammer and C.R. Zochodne. Anesthesia Research, Brigham & Women's Hospital, Boston, MA 02115.

Single axonal units from isolated frog sciatic nerve were recorded via suction electrodes which were stimulated using a train of 5-3 times the tonic threshold for the recorded unit) during exposure of the nerve to stable concentrations of the local anesthetic lidocaine. At [lidocaine] below the threshold for a tonic "ionic" conductance failure for above-threshold stimuli at rates >0.5 Hz, units conduced impulses at a slowed velocity but with 100% fidelity. Increasing the number of neighboring units firing to each stimulation burst by increasing the stimulus current by a factor of 10 further slowed the conduction velocity, or CV, in 28 of 28 fibers studied, and led progressively to a fall in CV of conduction. The impulse slowing began after the first impulse at the tonal intensity and then progressively increased over about 30 s. Slowing or block also persisted for at least 30 s after the stimulus intensity was returned to baseline intensity or failure was observed without lidocaine. Instead, the tonal stroke intensity with [lidocaine] = 0 increased by 10% (reduced latency by 0.3-0.5 ms).

The recovery time for latency to return to the tonic latency, recorded prior to the tonal stroke intensity, depended on the number of stimuli given at the higher intensity. If the intensity was maintained at the high level, activating more neighboring fibers, then the slowing or block was likewise maintained. Repeated activation of conduction bursts of 8 impulses at tonal intensity produced conduction failure that persisted for more than 30 min. The cumulative slowing and block was observed only for fibers that had conduction failure since in cases where multiple fibers were recorded, the ones near block did not slow when the intensity was increased. Because of the 30 s accumulation and recovery times and because of a transient increase we observed in the CV after tetanic conditioning, which was known to result in post-tetanic hyperpolarization, we attribute the modulation of block to a potentiation of lidocaine blocking by a depolarization of the affected axon by its activated neighbors, possibly resulting from increased axonal potassium.

477.11
INTERLEUKIN-1β IL-1β INHIBITS SYNAPTIC TRANSMISSION IN THE BASOLATERAL AMYGDALA (BLA). E-J. Yu, D. Rainnie and P. Shimnick-Gallagher*. Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77555

IL-1β, a cytokine, has central actions including anticonvulsant activity. We analyzed IL-1β effects on synaptic transmission using intracellular recording in the BLA, a nucleus involved in limbic epilepsy. In the BLA, stimulating the stria terminalis (ST) or lateral amygdala (LA) elicits excitatory post synaptic potentials (EPSPs) and fast- and slow-inhibitory post synaptic potentials (IPSPs) via both feed-forward and direct pathways. ST- and LA-evoked EPSPs were reversibly depressed by IL-1β (2 ng/ml). Fast- and slow-IPSPs evoked by stimulating the ST and LA were also reduced or completely blocked suggesting direct and indirect effects on GABAergic transmission. Furthermore, the reversal potential for the IPSP was not altered by IL-1β. Analysis of input-output relationships indicated that the inhibitory effect of IL-1β on synaptic transmission was not overcome by increasing the stimulus intensity. We tested whether IL-1β inhibition was due to a pre- or postsynaptic action by applying agonists exogenously. Muscimol responses were not altered by IL-1β suggesting presynaptic inhibition of the fast IPSP. Responses to AMPA were slightly decreased but the ST- and LA-evoked EPSPs were decreased to a greater extent; IL-1β effects on AMPA responses were not reversible. These data suggest that IL-1β inhibits excitatory and inhibitory transmission at a presynaptic site and provide evidence that IL-1β, a mediator of the immune response, has an inhibitory effect on synaptic transmission in CNS neurons. Furthermore, these results suggest that the BLA nucleus may be a neuronal substrate for interactions between the immune and central nervous systems.(supported by NS 29265).

477.13
EXTRACELLULAR MESSENGERS PRODUCED BY ECTOPROTEIN KINASE IN DEVELOPING NEURONS. H. Yang, M.V. Hogan and Y.1. Endlich. CUNY at Staten Island, NY 10301 and the Biology Doctoral Program of CUNY Graduate School.

Ecto-Protein kinase (ePK) utilizes extracellular ATP to phosphorylate proteins localized at the external surface of the neuronal plasma membrane. To determine the role of ePK in neuronal development, we investigate primary neurons cultured from the telencephalon of 7-day chick embryos. Phosphorylation reactions are carried out with cells attached to 48-well plates. For ePK assays we add γ-32P-ATP (0.1 μM, 15Ci/mM) and incubate for 10 min. To incubate intracellular proteins an equivalent dose (15Ci/mM) of [32P]ATP is added, with [32P]ADP at 2 min. We find that two proteins with apparent MW of 11.7K and 13K became rapidly phosphorylated upon addition of γ[32P]-ATP to the medium, but were not labeled at all even after 1-2 hrs incubation with [32P]. These proteins were identified as the substrates of ePK. The extracellular phosphorylation of these proteins was found to peak at the onset of rapid neurogenesis. In the present study, we expose the cells in three times with Krebs-Ringer buffer just prior to the ePK reaction, to remove all soluble components. Immediately after 10 min incubation with γ[32P]-ATP, the medium was separated from the attached cells by centrifugation (150,000 x g, 90 min). The 11.7K and 13K phosphorylated substrates of ePK were found in the soluble fraction. Thus, during the extracellular phosphorylation reaction these phosphorylated proteins detach from the cell surface. In developing neurons, this process may provide a unique, novel means of controlling growth and function of target neurons, and for interaction with components of the extracellular matrix.
478.3
CONVERGENT REGULATION OF BRAIN SODIUM CHANNELS BY CAMP-DEPENDENT PROTEIN KINASE AND PROTEIN KINASE C. Ming Li*, James W. West, Todd Scheuer, William A. Catterall. Dept. of Pharmacology, University of Washington, Seattle, WA 98195.

Direct application of the catalytic subunit of camp-dependent protein kinase (cAMP-PK) and ATP to the cytoplasmic surface of rat brain Na channels is excised in-out our membrane patches reduces channel activity. Protein kinase C (PKC) phosphorylation of Ser1506 in the conserved intracellular loop between domains III and IV of the alpha subunit slows inactivation and allows reduction of conductive activity by phosphorylation at another site in the loop between domains I and II (Numann et al., preceding abstract). Since phosphorylation at Ser1506 is necessary but not sufficient for reduction of Na current by PKC, we examined whether it might also control reduction of channel activity by phosphorylation of Ser1506 directly. PKC, no current recorded from Chinese hamster ova cells stably expressing Type IIa sodium channels with the mutation Ser1506Ala were unaffected by application of cAMP-PK and ATP. Application of cAMP-PK and ATP to 6 patches containing wild-type channels reduced the current by 40% in the same series of experiments. The reduction in wild-type channel activity by cAMP-PK cannot be due to phosphorylation of Ser1506 since: 1) this site is not phosphorylated by cAMP-PK in biochemical experiments and 2) phosphorylation at this site by PKC causes only slowing of inactivation of Ser1506 by PKC. No current recorded from Chinese hamster ova cells stably expressing Type IIA sodium channels with the mutation Ser1506Ala were unaffected by application of cAMP-PK and ATP. The PKC consensus site at Ser1506 was converted to a cAMP-PK consensus site by conversion of Lys1507 and Lys1508 to Glu. In contrast to wild-type Na currents are slowed and reduced by cAMP-PK and ATP. The effects of cAMP-PK are similar to PKC in wild-type and consistent with the conclusion that phosphorylation of Ser1506 by PKC slows Na channel inactivation and regulates the reduction of Na channel activity by both PKC and cAMP-PK.

478.4
MUTAGENESIS OF THE RAT BRAIN SODIUM CHANNEL PROTEIN KINASE A (PKA) PHOSPHORYLATION SITES. R.D. Smith* and A.L. Goldin, Dept. of Pharmacology & Microbiology Genetics, U. California, Irvine, CA 92717.

We have previously described that substitution of the cAMP binding protein kinase A (PKA) in Xenopus oocytes results in an increase in sodium currents expressed from the rat brain type I sodium channel clone. We have now used site-directed mutagenesis to determine the current control by phosphatase activity. Protein kinase A (PKA) phosphorylation of the sodium channel alpha-subunit, Ser1506 was the result of direct phosphorylation of the sodium channel alpha-subunit. The sodium channel has five consensus PKA phosphorylation sites located in the linker region between domains I and II. It is possible that sites were either eliminated by replacing the serine residues with alanine, or were disabled by replacing the serine residues with aspartate. All five of the serine to alanine substitutions resulted in functional changes in sodium currents, with the most significant effect being a decrease in current. The voltage-dependent properties and inactivation kinetics of all of the mutants were similar to those of the wild-type channel. All five of the mutant channels also showed some degree of the following PKA stimulation; but the magnitude of the effect was quite variable and dependent on the specific mutation. Four of the five serine to aspartic acid mutations have thus far been expressed. The current properties of these mutated channels were tested by each position is sufficient for normal sodium channel function.

478.6
CLONING AND SEQUENCE OF A PUTATIVE SQUIRREL SODIUM CHANNEL cDNA AND A PROPOSED TERTIARY Structure MODEL. C.B. Mile and P.T. Catterall. Electrotechnical Laboratory, Tsukuba, Ibaraki 305, Japan.

With polymerase chain reaction and recombinant DNA techniques, we have cloned a complementary 4.9 kbp cDNA which seems to encode a sodium channel protein of the optic lobe of squid Loligo bleekeri. The total number of amino acid residues deduced from the cDNA is 1,522, about three-fourths of that of rat brain I, II and III (each being about 2,000) and an even proportion in eel (1,820). The estimated molecular weight of the squid sodium channel protein is 174,105 dalton. The squid sodium channel is basically quite similar to that of vertebrates, and consists of four domains I, II, III and IV, each containing five hydrophilic segments (S1, S2, S3, S5 and S6), one characteristic segment with strong positive charge (S4), and short intervening sequences. Both a negative charge and a positive one in S2, and a negative charge in S3 as well, are entirely conserved at identical positions in all the domains; only the positive charges are serially repeated at every third position in I-IV. On the basis of the amino acid sequence data, we propose a tertiary structure model of the squid sodium channel.
478.7
CONTRIBUTED TALK


Previous work by our laboratories has shown that SM2 is the predominant Na+ channel gene expressed by the neuronal derivatives of the RT4 cell line family. Since the RT4 family was derived by a retroviral transformation, we presented the possibility that the SM2 Na+ channel gene might be important in vivo in the rat CNS. In addition our findings suggested that the RT4 system may derive from dorsal root ganglia (DRG) since small cell neurons from rat DRG have high levels of Na+ current and the only known Na+ channel gene thought to encode a TTX-resistant channel is the SM2 gene. We are pursuing two lines of investigation: 1) to ask whether other markers of small cell DRG neurons are expressed by the RT4 neuronal cell types. We have examined the expression of SM2 from newborn rats by RNase protection assay and detected low levels of SM2 mRNA. We are currently in the process of examining DRG by in situ hybridization histochemistry to determine which DRG cell type(s) express SM2. We have also determined that the RT4 neuronal derivatives do not express the three neurofilament proteins, as is the case for small cell DRG neurons. Small cell DRG neurons express the intermediate filament protein peripherin and we are presently examining whether the RT4 neuronal derivatives express peripherin.

478.8
CONTRIBUTED TALK

THE SODIUM CHANNELS OF THE CYANEID JELLYFISH Cyanea capillata. A HIGH MOLECULAR WEIGHT PROTEIN WHICH BINDS THE CALCIUM CHANNEL BLOCKER AZIDOPINE. Peter A. V. Anderson and John A. Schetz. Dept. of Neurosciences and the Whitney Laboratory, Univ. of Florida, 9505 Ocean Shore Blvd, St. Augustine, Florida 32080.

Previous electrophysiological studies of neurons from the jellyfish Cyanea capillata (Anderson 1987, J. Physiol., 396, 86P) revealed the presence of a fast Na+ current which is completely insensitive to the classic Na+ channel blocker, TTX, but it is blocked by the L-type Ca++ channel blockers known as dihydropyridines (DHP). Since Cyanea Ca++ currents are insensitive to DHPs, this unusual combination of channel pharmacology in Cyanea allowed biochemical analysis of the Cyanea Na+ channel. Isolated membranes from nerve-rich tissue in Cyanea were digitonin-solubilized, applied to an azidopine affinity column and eluted with drug. Column eluates were concentrated and size fractionated by SDS-PAGE. Silver staining revealed a distinct protein band with a molecular weight of approximately 200 KDa only in column fractions eluted with a concentrated DHP solution. This size is consistent with that deduced from a putative Na+ channel cDNA isolated from Cyanea.

Special thanks to Dr. H. Glossman for providing Azidopine. Supported by NSF Grant BNS 9109155 (PAA).

478.9
CONTRIBUTED TALK


Voltage-gated sodium channels are important contributors to the intrinsic excitability properties of CNS neurons. The aim of this study was to determine whether the relative expression of human brain sodium channel (HBSC) subtypes I and II is altered in epileptic brains compared to those of normal controls. To this end, we show that the ratio of these two subtype mRNAs varies markedly between different normal brain regions along the neuroaxis, but for a given region the value is generally consistent between individuals (Lee et al., FEBS Lett., in press). Epileptic tissue was obtained at surgery from patients undergoing treatment for intractable seizures. Postmortem tissue was obtained from brain regions, age and sex were used as normal controls. Following RNA extraction, the relative amounts of type I and II sodium channel mRNA was compared in these tissues using a modification of the ligase detection reaction as described previously (Lu et al., FEBS Lett., in press). This protocol, combining high sensitivity and high specificity, proves useful for measurements of highly homologous messages such as HBSC I and II, since specificity is provided at the level of single nucleotide differences.

Compared to normal postmortem controls, the data reveal an increased ratio of the I:II ratio in epileptic tissues, with moderate relative increases in HBSC I-specific transcripts. Additionally, a 13:9 ratio of 0.43 was determined for normal temporal lobe, a region not previously characterized with respect to sodium channel mRNA expression. These results suggest a potential correlation between epileptic activity and alterations in the expression of subtypes I and II of the human brain sodium channel. Moreover, these results may lend insight into the control of neuronal excitability and its contribution to the state of hyperexcitability in epileptic tissue.

478.10
CONTRIBUTED TALK

FOUR FORMING REGIONS ON VOLTAGE-SENSITIVE POTASSIUM AND SODIUM CHANNELS SHARE A COMMON SECONDARY STRUCTURAL MOTIF TARGETED BY STRUCTURALLY SIMILAR SCORPION TOXINS. John A. Schetz, Department of Neuroscience and The Whitney Laboratory of the University of Florida, Gainesville, Florida 32607.

A secondary structure within the putative SS-56 pore-forming regions of voltage-sensitive K+ and Na+ channels was compared and quantified with a statistical metric (SCH (C) version 6.10). The metric predicts that the a-scorpion toxin receptor region mapped to SS-556 on rat brain II Na+ channel and the charybotoxin receptor region mapped to SS-56 on the Shaker A K+ potassium channel are structurally similar (overall fit = 92%), even though their amino acid sequences are sparsely homologous (18%). The striking secondary structural similarity between K+ channel and Na+ channel sequences in comparable SS-56 regions demonstrates that scorpion peptide toxins target structurally similar channel receptor sites. This result, in combination with the fact that the peptide ligands, charybotoxin and a-scorpion toxin Aa II, are themselves structurally similar (Bontems et al. 1991, Science, 254, 1521-1523), invites the hypothesis that venomous peptide ligands of comparable structure bind "pore"/receptor regions of comparable structure.

478.11
CONTRIBUTED TALK

BIOCHEMICAL EVIDENCE THAT THE TTX-INSSENSITIVE SODIUM CHANNEL IN THE JELLYFISH Cyanea capillata IS A HIGH MOLECULAR WEIGHT PROTEIN WHICH BINDS THE CALCIUM CHANNEL BLOCKER AZIDOPINE. Peter A. V. Anderson and John A. Schetz. Dept. of Neurosciences and the Whitney Laboratory, Univ. of Florida, 9505 Ocean Shore Blvd, St. Augustine, Florida 32080.

Although the literature on saxitoxin (STX) binding in the CNS is abundant, there is little data on the neuroanatomical distribution and STX binding properties in various brain nuclei. In the present study, we examined in detail the saturation profiles of STX binding at several CNS levels using a broad concentration range of STX (up to 64 nM) and mapped STX sites at low (2 nM) and high (15 nM) concentrations of STX. Since the density of STX binding sites (nM) may be an important factor that determines resistance to anoxia, we also compared rat to cattle CNS. We found that 1) Scatchard plots were linear with Kd values around 1 nM for a region of the brain including most cortical areas; 2) in some nuclei, especially in the brainstem, these plots showed an increase followed by a decrease in Bound/FREE Versus Bound; 3) STX binding density was heterogeneous at both binding concentrations with a much higher density in rostral areas than in the brainstem; and 4) saturation patterns in the turtle brain were similar to those of the rat, but STX binding density was much lower (several to 10 fold) than in the rat. We conclude that STX binds mainly to a high affinity site and 2) positive cooperativity occurs with STX binding in some areas. Some areas have shown that Na+ plays an important role during anoxia, we speculate that low binding density in the turtle brain may be partly at the basis of its hypoxic tolerance.

478.12
CONTRIBUTED TALK


Previously, a high-affinity site for STX was mapped to a site in the rat brain similar to the binding site for benzodiazepines. Using this approach, we have shown that benzodiazepines at high concentrations bind to a single site that is distinct from the TTX-binding site in the rat brain. In the present study, STX binding was measured in several rat brain regions using a variety of concentrations of STX ranging from 0.1-50 nM, and the data were analyzed using a one-site binding model. The results showed that there are at least three distinct binding sites in the rat brain, each with a different affinity for STX. The binding of STX was also measured in several areas of the turtle brain. The binding of STX was highest in the medulla oblongata and the spinal cord, with lower binding in the brainstem and cerebrum. The binding of STX was also measured in several areas of the turtle brain. The binding of STX was highest in the medulla oblongata and the spinal cord, with lower binding in the brainstem and cerebrum. The binding of STX was also measured in several areas of the turtle brain. The binding of STX was highest in the medulla oblongata and the spinal cord, with lower binding in the brainstem and cerebrum. The binding of STX was also measured in several areas of the turtle brain. The binding of STX was highest in the medulla oblongata and the spinal cord, with lower binding in the brainstem and cerebrum. The binding of STX was also measured in several areas of the turtle brain. The binding of STX was highest in the medulla oblongata and the spinal cord, with lower binding in the brainstem and cerebrum.

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Dorsal root ganglion neurons acutely dissociated from 3-12 day old rats express TTX-sensitive (TTX-S, 1 nM) and TTX-resistant (TTX-R, Kp = 100 μM) sodium channels, which have been shown to differ in their biophysical and pharmacological properties. Leiurus quinquestratus, scorpion venom and sea anemone toxin ATX-II (1-100 nM) exerted no significant effects on TTX-S and TTX-R currents. Tissue dissociation procedures (collagenase-dispase, papain, trypsin, and mechanical dissociation) were systematically examined, each generating the same results. The binding region(s) of both toxins may thus be absent in these cultures. α-Dihydrorayanotoxin (GTX) and deltamethrin, however, each exerted differential effects on TTX-S and TTX-R channels. GTX (100 nM-10 μM) shifted TTX-S channel activation voltage slightly (~2.5 ± 4.1 mV, n=6), without affecting reversal potential. TTX-R current activation was minimally affected (~3.5 ± 4.3 mV, n=8) whereas the reversal potential was dramatically shifted (~17.9 ± 9.1 mV). Deltamethrin (1-100 nM), a type II pyrethroid, shifted activation and reversal potentials of the TTX-S current, without a significant effect on tail current. TTX-R tail current, however, was greatly prolonged, with rising values of greater than 10 seconds. The differential properties of the TTX-R and TTX-S sodium channels continue to be of interest in CNS development and drug interactions. Supported by NIH grants R31 MH09389 and R01 NS14143.

SODIUM CHANNELS

478.15 DIFFERENCES IN THE NEUROEXCITATORY ACTIONS OF SODIUM CHANNEL SPECIFIC NEUROTOXINS IN RAT AND TROUT BRAIN SYMPATHEICOS. J.T. Epstein, K.A. Plejtey, J.L. Baumussen and A.R. Holman, Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

The effect of pyrotoxin insecticides and other sodium channel specific neurotoxins on neuronal excitability were investigated in rat and trout brain synaptosome preparations using a membrane permeant, lipophilic cation ([3H]-tetraphenylphosphonium (TPP+)). Concentration-dependent and tetraethylammonium (TEA)-sensitive decreases in TPP+ accumulation indicative of membrane depolarization were produced by acridine (Acn), veratridine (Vid), scorpion (Leiurus quinquestratus) venom (ScV) and type I and II pyrotoxins in both species. Acn, Vid and ScV were more potent and efficacious membrane depolarizing agents in rat synaptosomes than in trout synaptosomes. Type II (deltamethrin, cypermethrin and fenvalerate) pyrethroids produced similar depolarizing responses in rat and trout synaptosomes, however, the R.I., or asomer of deltamethrin which had no effect on membrane potential in rat synaptosomes depolarized trout synaptosomes. In addition, the type I pyrethroid, permethrin, exhibited significantly greater efficacy in trout synaptosomes compared to several sodium channel specific neurotoxins and suggest that some of the neurotoxin binding domains of the voltage-sensitive sodium channel in trout brain differ from those in mammalian brain. The hypersensitivity of fish to the neurotoxic actions of pyrethroid insecticides may be related to these differences. (E500006 and E5001959)

478.17 EFFECT OF SODIUM CHANNEL MODULATING DRUGS ON LOSS OF EPSFS FROM HYPOTHYPOGLYCEMIA IN RAT HIPPOCAMPAL SLICES IN VITRO. C.P. Tasson, J.L. Tatter, P.C. Hare. Dep. of Physiology and Pharmacology, Wright State University, Dayton, OH 45419.

Previous studies with phenytoin, lidocaine and tetrodotoxin in various models of ischemia have shown no protection, suggesting the involvement of voltage-sensitive sodium channels in ischemic brain damage. We investigated this hypothesis with several modulators of voltage-sensitive sodium channels using rat hippocampal slices under conditions that mimic ischemia. Hippocampal slices were incubated under control conditions and then subjected to a 15 min period of "ischemia" from depolarization produced by an increase in extracellular K+ (from 3 to 60 mM). This treatment caused a loss of synaptic potentials over 2-5 min and a sudden negative shift in extracellular voltage corresponding to loss of ion homeostasis after about 8 min. Return of normal medium failed to recover EPSFs in any of 96 control experiments.

However, treatment with phenytoin (20 or 50 μM), lidocaine (50 or 100 μM), carbamazepine (50 μM), or verapamil (10 μM) significantly delayed the onset of negative voltage shifts and increased the number of slices that recovered EPSFs. These neuroproactive effects were seen at drug concentrations that did not alter synaptic action potential amplitude, indicating that sodium channels were modulated but not blocked. Nimodipine at a concentration (1 μM) that modulates L-type voltage-sensitive calcium channels did not alter synaptic action potentials. DIDS and D-APV, modulators of voltage-dependent sodium channels by anticonvulsant or local anesthetic drugs may be beneficial for treatment of cerebral ischemia.
478.19 ANTIMYOTONIC ACTIVITY AND USE-DEPENDENT BLOCK OF SKELETAL MUSCLE Na+ CHANNELS BY ENANTIOISOMERS OF TOCAINIDE AND MEXILETINE. A. De Luca*, C. Panchal, Y. Tongiorgi, S. H. Bryan, and D. C. Cameron. Dept. of Pharmacology and Pharmacoeconomics, Fac. of Pharmacy, Univ. of Bari, Italy and Dept. of Pharmacol. and Cell Biophysics, Univ. of Cincinnati, USA.

The use dependence of class I antiarrhythmic drugs as tocainide, as racemate, and as its individual enantiomers on rat muscle sodium channels has been studied. The high affinity interaction during slow inactivation states of Na+ channels (De Luca et al., Neurosci. Lett., 344, 75-78, 1998) in mammalian muscle cells has been described to be stereospecific (Tricario et al., Pflügers Arch., 418: 500, 1991). To clarify the role of stereospecificity in the use-dependence of these drugs, the ability of enantiomers of tocainide and mexiletine to solve abnormal membrane excitability was evaluated "in vitro", by means of two intracellular microelectrodes technique, on rat extensor digitorum longus (EDL) muscle, made myotonic by previous ionization with 50µM atracurium-9-carboxylic acid (9AC), and on external intercostal muscle fibers from congestiously myotonic goats. On rat EDL, 30µM (S)-tocainide completely antagonized the 9AC-induced hyperexcitability, whereas 10µM (R)-tocainide inhibited only 25% the myotonic repetitive firing. On the other hand, 50µM of (R)- and (S)-tocainide inhibited 9AC hyperexcitability by 70% and 60%, respectively. On myotonic goats, 10µM (R)-tocainide fully restored normal membrane excitability, whereas 100µM (S)- was almost ineffective. These compounds were also tested on Na+ currents recorded from goldfish semimembranosus muscle fibers by means of triple Vaseline gap voltage clamp. External application of 500µM of either (R) (+)- and (S)-tocainide blocked Na+ currents by 25-50%. Similarly, both enantiomers of mexiteline had an EC50 of about 300µM. Either eosin or racemates of mexiteline and mexiletine were strongly use-dependent, i.e., the further cumulative reduced peak Na+ current (up to 70%) upon repetitive stimulation at 2Hz frequency. The present data suggest that the presence of a weakly stereospecific receptor (as in frog muscle), does not impair the use-dependence of Na+ channel blockers. However, it is the presence of highly stereospecific site, as in mammals, modulates use dependence of drugs with pronounced stereoselectivity, as tocainide and this might have important therapeutic implications. (Granted by CNRS 91.0026 and Telethon-Italy, 1991).
478. 23  
NON-NA (ACTIVATING), TTX-SENSITIVE Na⁺ CONDUCTANCE IN RAT OPTIC NERVE AXONS: POSSIBLE PHYSIOLOGICAL AND PHARMACOLOGICAL ROLES.  
Dept. Neurology, Yale School of Medicine, New Haven, CT 06510.  
Sodium channels provide the rapid depolarization underlying axonal action potentials. Classical channel kinetic models describe channels quickly and completely inactivate at depolarized potentials, contributing to rapid termination of the action potential. TTX-sensitive Na⁺ spicules (ROP) as a model to study the ionic mechanisms of anoxia in current meylitined axons. Anoxic injury in the RON is critically dependent on Na influx via a TTX-sensitive Na conductance. However, conventional Na channels should be inactive at the depolarized membrane potentials that occur in anoxic tissue. We therefore hypothesized that a non-inactivating Na conductance must exist, which mediates pathological Na fluxes during anoxia. To test this prediction, we studied RONs with a modified griseofulvin technique. The recorded DC potential is a reliable fraction of the true complex axonal resting potential (VR), behaving as a linear function of transp-  
membrane voltage (VR=4 mV at 10 mV; VR-10 mV at 10 mV) and at two levels of  
depolarization (KJ=15 and 40 mV); at [K]=40 mV, resting potential decreased to  
44% of control. A TTX-induced hyperpolarization was observed at each value of  
[K], indicating that a fast, non-inactivating Na conductance exists at rest and 
at depolarized membrane potentials in RON axons. PKP-2 PNa were estimated to be  
50 22.1 and 17.8 at [K]=15 and 40 mV, respectively. Following the 
TTX-induced hyperpolarization, VR began to depolarize. We suggest that the  
depolarization was partly due to inhibition of the Na K-ATPase secondary to  
depolarization of intracellular Na. We conclude that meylitined axons of the RON possess a TTX-sensitive, non-inactivating Na conductance. This conductance may function as a source of intracellular Na for the Na-K-ATPase under physiological conditions. Under pathological conditions such as anoxia, this conductance plays a central role in  
modulating irreversible injury by admitting Na, raising (Na) and driving the Na-Ca 
exchange to import damaging quantities of Ca. This non-inactivating Na conductance may have unique pharmacological properties and its blockade may  
protect CNS while matter from anoxia without abolishing electrogenesis (RES was supported by a Convervational Fellowship from the Medical Research Council of Canada).  

478. 27  
SPONTANEOUS TTX-SENSITIVE Na⁺ CURRENTS IN RATTEN SENSORY NEURONS MAY INVOLVE ALL-OR-NONE Ca²⁺ TRANSIENTS.  
Lab. of Neurophsiology, NNIDS, NIH, Bethesda, MD.  
Whole-cell patch recordings in the presence of 1-3 μm tetrodotoxin (TTX) revealed spontaneous transient inward currents (STICs) in cultured (1-3 weeks) dorsal root ganglion cells dissociated from 19-20-day-old rat embryos. The frequency of STICs was below the threshold of detection before the peak amplitude. Removal of extracellular Ca²⁺ decreased their frequency to 7 to 70% of control, but did not eliminate STICs. Addition of 2.5 mM Mgs²⁺ increased their frequency to 2.5 times of control, but preserved the preferred-amplitude characteristics. Replacement of extracellular Na⁺ with N-methyl-D- 
glucamine reduced the frequency of STICs, shifted the distributions of peak amplitudes to lower levels, prolonged their time to peak, and decreased their rate of rise. Extracellular Na⁺ free reversibly eliminated STICs. When a membrane permeable Ca²⁺ chelator, BAPTA-AM, was added to the bath, the frequency of STICs decreased progressively to 6% of control. The properties of STICs suggest that these TTX-resistant Na⁺ dependent currents may be activated by all-or-none Ca²⁺ transients released from intracellular stores.  

478. 28  
Action potentials in cultured hippocampal neurons from the trisomy 16 (T16) mouse have a slower depolarization rate than control neurons. This is likely due to differences in the voltage dependent sodium current. Primary cultures of hippocampal neurons were prepared from control and T16 mouse fetuses at day E15-16. Whole cell patch clamp recordings were performed from neurons 2-4 week old neurons. The extracellular medium included 130 NaCl, the intracellular medium included 5 NaCl. Currents were evoked with a 10 ms pulse in 10 ms steps between -50 mV to 90 mV from a holding potential of -40 mV. The mean membrane resistances were approximately 500 MΩ, the capacitances approximately 30 pF and the reversal potentials approximately +80 mV. None of these values were significantly different between control and T16 neurons. The mean maximum inward current was -125 pA/pF for control neurons (n=16) and -62 pA/pF for T16 neurons (n=16) (significant at p < 0.01), and was abolished by 1μM TTX, but unaffected by 2 μM CdCl₃. This indicates that the current is a voltage-dependent sodium current, and likely explains the reduced depolarization rate of the action potential.
478.31 ELECTROPHYSILOGICAL CHARACTERIZATION OF CATHA CELLS - A NEW CATECHOLAMINERGIC CNS CELL LINE. M. Lazareno, D. A. Chakrabarty, K. Dunlap. Neuroscience Program, Tufts Univ. Sch. of Med., Boston, MA 02111. A CNS cell line (CATHA) was previously derived from a brain tumor from a transgenic mouse in which V540 T antigen was under the transcriptional control of rat tyrosine hydroxylase 5' flanking sequences. The cells synthesize catecholamines and have neurotransmitter and sympathophin proteins whereas glial fibillary acidic protein is absent suggesting that the CATHA cells are neuronal in origin. We have evidence for the presence of voltage-dependent ion channels by whole cell voltage-clamp recording. When CATHA cells are depolarized from a resting potential of -80 mV to between -40 and -20 mV, a fast-inactivating inward current is observed. Peak current is reached within 2 ms and is followed by an exponential inactivation (1.6 ms). Current-voltage relations show a maximum current near 0 mV. This current resembles that of the voltage-dependent Na+ channels in other preparations. This was further confirmed by ion substitution experiments in which external NaCl was replaced with N-methyl-D-glucamine (NMDG); this substitution eliminated the current. In addition, 250 mM tetradotoxin (TX) blocked the current in a reversible manner. These findings suggest that CATHA cells express voltage-gated Na+ channels. When depolarized to -40 mV, a longer lasting inward current is observed in approximately 40% of the CATHA cells. This current is blocked by 100 μM cadmium and is carried predominantly by cadmium. In other CATHA cells a long-lasting, more slowly activating outward "K+" like current is seen. Therefore, these transformed cells appear to possess voltage-gated sodium channels, calcium channels, and possibly potassium channels.

478.32 NA+/K+ ATPase INHIBITORS BLOCK PALTOXYL INDUCED INACTIVATION OF IONIC CURRENTS IN FROG MUSCLE MEMBRANE. M.B. Rodriguez de Salazaga, S. Ortiz-Miranda and G. Escalona de Motta*. University of Puerto Rico Dept. of Biology and Institute of Neurobiology, San Juan, Puerto Rico. Paltoxon (PTX), isolated from zoanthids of the genus Paltozon (Cnidaria) and considered one of the most potent marine toxins, has been proposed to increase Na+ permeability of cell membranes through a mechanism involving the Na+ K+ ATPase. In vivo inhibition of Na+ K+ ATPase. However, the inhibitory action of this glycoside was dependent on their chemical structure, monoglycosides being the most active inhibitors. This results confirm a relationship between the depolarizing effect of this toxin and the activity of the membrane Na+/K+ ATPase. (Work was supported by NIH grants GM168102, NS07464 and NOAA, USDC Sea Grant College Program at UPR).

CALCIUM CHANNEL MOLECULAR BIOLOGY

479.1 CLONING OF THE α1 SUBUNIT OF A VOLTAGE-DEPENDENT CALCIUM CHANNEL EXPRESSED IN THE ELECTRIC ORGANS FROM NARCINE BRIASISCUISI. M. Philip, J. Lin, C. Hastings, L. Stiib, S. Azhar, J. Forc, J. Ramachandran, and J. Bell. Neuron Corporation, 3760 Haven Avenue, Menlo Park CA 94025. The electric organ of the fish Narcine brasiliensis is a rich source of molecules such as calcium channels involved in neuronal communication. Using two oligonucleotides from motif IV and the carboxy tail of the rabbit skeletal muscle calcium channel as probes, we cloned a cDNA encoding an electromotor nucls as template, and PCR, we cloned a 570 bp fragment and subsequently used it as a probe to screen a cDNA library made from electromotor nucls. From the library, we isolated several overlapping clones covering almost the full length of the message. Upon sequencing, these clones showed a high similarity to Class D channels (Stuchl, T.P. et al., (1990) Proc. Natl. Acad. Sci. Vol 87, 3391; Williams, M.E. et al., (1992) Neuron, Vol 8, 757). Human and fish genes highly conserved with 79% identity overall at the amino acid level. There is almost complete conservation of transmembrane domains and significant divergence in the intracellular regions between motifs I and II and between motifs II and III. In preliminary results, the C-terminal region of our fish clone is relatively short and diverges completely from the human clone 177 residues from the last transmembrane domain, perhaps suggesting alternate splicing in this region. It appears that the subtypes of calcium channels arose early in vertebrate evolution and are highly conserved among different species. We also obtained a presumptive splice variant, unique from other published sodium or calcium channels, which has two in-frame deletions of 129 bases and 66 bases corresponding to the IVS3 and IVS5 transmembrane domains.

479.3 CLONING AND CHARACTERIZATION OF A NOVEL α1 SUBUNIT OF DROPSOPHILA Ca2+ CHANNEL. W. Zheng, G.P. Feng, D.F. Eberl, D.J. Trickle* and L. M. Hall. Dept. of Biochemical Pharmacology, School of Pharmacy, SUNY/Buffalo, NY 14260. The most abundant Drosophila head membrane channels have different pharmacological properties than those of vertebrate L-type Ca2+ channels. These Drosophila Ca2+ channels have high binding affinity for phyllolamines, but are insensitive to dihydropyridines and to benzothiazepines whereas the vertebrate L-type channels are sensitive to all three classes of the Ca2+ channel antagonists. We report here a complete sequence of the Ca2+ channel α1, subunit cloned from a Drosophila head cDNA library. This cDNA encodes a deduced protein estimated to contain 1964 amino acids. It exhibits 79.5, 72.2 and 71.2% sequence similarity to the α2 subunit expressed in rat brain (D), rabbit skeletal muscle and heart respectively. A comparison of the Ca2+ channel sequences from Drosophila and vertebrates will be presented which provides important insight concerning the nature of phyllolamine and dihydropyridine binding sites. Northern analysis shows that this α1 subunit mRNA is expressed as a single size class in leg (9.5kb) and body (9.6kb) but as three size classes (9.5, 10.2 and 12.5) in head. This gene has also been localized on the left arm of chromosome 4 by in situ hybridization. Mutant analysis will define the physiological developmental role of this channel in the whole organism. (Supported by NIH grants HL16003, HL39369 and GM24850-02).
479.5 MOLECULAR CLONING AND REGIONAL EXPRESSION OF A RAT BRAIN CALCIUM CHANNEL L-P Type Subunit. J.P. Szurszewski, T.H. Purves, D.J. Dubel, W.J. Tontonoz, T.Y.B. Starr, S.R. Vishteg and T.P. Szurszewski. Biotechnology Laboratory and "Division of Neurological Sciences, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

Voltage-gated Ca channels are heterologeric complexes that include an alpha-1 subunit that serves both as a voltage sensor and ion conduction channel. In addition, exogenous expression studies demonstrate that separate subunits (alpha-2, beta and gamma) alter the physiological properties of the alpha-1 subunit. In the nervous system, the exact subunit composition of different neuronal Ca channel types has not been described and it is not yet known whether all Ca channel alpha-1 subunits are associated with auxiliary subunits. Utilizing the skeletal muscle alpha-2 subunit as a probe we have isolated a 4.4 kb cDNA from a rat brain library. The cDNA encodes a 10% amino acid protein (123 kDa) predicted molecular mass) that shows 93% and 94% amino acid identity to rabbit skeletal muscle and human brain Ca channel alpha-1 subunits, respectively. The cellular localization of the alpha-2 subunit expression was examined in adult rat brain using a pigment hybridization with antisense oligonucleotides.

Antibody studies reveals that the alpha-2 subunit is highly expressed in a subset of neurons in the cerebellum, olfactory bulb, hippocampus and cortex. The expression of the brain alpha-2 subunit does not correlate exactly, with the expression of Ca channel alpha-1 subunits and suggests that only a subset of neuronal Ca channels are associated with an alpha-2 subunit.

479.6 cDNA CLONING OF A CALCIUM CHANNEL HIGHLY EXPRESSED IN THE HIPPOCAMPUS. L-C. Chong, L.G. Moon and T.J. Trimmer. Biotechnology Laboratory, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

Screening of a cDNA library constructed from whole rat brain RNA identified four major subtypes of Ca channel alpha-1 subunits (classes A, B, C, and D). DNA sequencing of the four Ca channel classes A and B alpha-1 subunits encode distinct L-type Ca channels while the class A and B alpha-1 subunits are likely to encode P and N type Ca channels, respectively. To identify Ca channels that may be involved in processes such as long-term potentiation, we have utilized the polymerase chain reaction (PCR) to amplify Ca channel sequences expressed in the hippocampus. Degenerate oligonucleotides were used to amplify hippocampus RNA isolated from adult rats. The PCR products were cloned and analyzed by DNA sequencing. Of 18 clones examined, 15 were identified as previously characterized Ca channel alpha-1 subunits, while 3 encode a new class of Ca channel (designated class E). The deduced primary structure of the class E protein is most closely related to the brain class A and B alpha-1 subunits. Limited amino acid identity to the brain class C and D alpha-1 subunit suggests that it is close to E channel corresponds to an L-type Ca channel. Northern blot analysis shows that the class E Ca channel is encoded by an approx. 12 kb RNA that is expressed throughout the rat CNS and is most abundant in the hippocampus. This pattern of expression is distinct from previously cloned Ca channels and may reflect a unique physiological role for the class E Ca channel in the CNS.

479.7 CHROMOSOMAL LOCALIZATION OF MURINE GENES ENCODING α1, α2, and β-SUBUNITS OF THE DHP-SENSITIVE L-TYPE CALCIUM CHANNELS. L. Qin, L. Marks, H.-L. Kim, and K. Kazazk. LMB, NINDS, TGL, NCI, and LAMM, NIADDK, Bethesda, MD 20892.

Recent molecular cloning has indicated that a heterogeneous family of voltage-sensitive Ca²⁺ channels are expressed in mammalian brain, providing structural bases for the functional diversity of neuronal Ca²⁺ channels. As a first step toward understanding genetic bases for diversity of brain Ca²⁺ channels, we have begun mapping the genes encoding the α1, α2, and β subunits of the dihydropyridine (DHP)-sensitive L-type Ca²⁺ channels. Previously, we and others localized two of the α1 subunit genes (Cchα1 and Cchα2) on mouse and human chromosomes (Powell et al., Genomics 1991; 54:68-79; 1991; Chiu et al., Genomics 1991; 11:21-91, 1991). Here, we have determined the chromosomal location of the third subunit gene, Cchα3, which encodes the isoform predominantly expressed in skinned skeletal muscle. Analysis of the progeny of an inbred strain cross positioned Cchα3 at 3.3 cM proximal to the Peps-3 locus on Chr 1. In contrast to the α1 subunit which are encoded by 3 distinct genes located on three different chromosomes, the Cchα2 and Cchβ subunits are encoded by a single gene, located on Chr 5 and 11, respectively. Analysis of 5 alleles for genes in an interspecies cross between Mus and C57 mice shows that the α2 subunit gene, termed Cchβ2, is positioned at the centromeric end of Chr 5, with gene order centromere-Cchβ1-Cchβ2-Cchβ3-Cchβ4. Similarly, the gene for the β subunit is mapped on Chr 11 with the gene order centromere - Sacr - Cchβ1-Bap3. Our mapping data indicate that the DHP-sensitive Ca²⁺ channel genes are apparently dispersed in the mouse genome, unlike the Na⁺ channel whose genes are clustered on Chr 2.


The dihydropyridine (DHP)-sensitive, L-type Ca²⁺ channel from skeletal muscle consists of 5 polypeptide subunits (α1, α2, β, γ, and δ). The α1 subunit of the Ca²⁺ channel can form the functional Ca²⁺ channels in Xenopus oocytes. However, cojection of skeletal muscle α2-6 and β subunit mRNAs with the α1 subunit mRNA drastically changed the electrophysiologic characteristics of the expressed Ca²⁺ channels. These findings suggest that α2-6 and/or β subunits may play a modulatory role in regulating Ca²⁺ channel function. Previously, we cloned and characterized the molecular properties of the DHP-sensitive α1 Ca²⁺ channel and α2 and β subunits. Here, we report the primary structure of rat brain DHP-sensitive Ca²⁺ channel β subunit and identify the distribution of its mRNA. Two cDNA clones, BT11 and BT8, encoding the β subunit were isolated and characterized. The deduced amino acid sequence of BT11 cDNA is very similar to that of the rabbit skeletal muscle β subunit, showing 96% amino acid identity. The BT8 clone is a spliced variant of BT11 with deletion of a 45-amino acid fragment. The distribution of rat DHP-sensitive Ca²⁺ channel β subunit mRNA was examined in prenatal (E16, E19), postnatal (P0, P6, P12), and adult rat brains as well as whole-body sections of E19 embryos. In adult rat brain, large amounts of β subunit mRNA were found in the hippocampus, dentate gyrus, and medial habenula. A high level of DHP-sensitive Ca²⁺ channel β subunit transcript was already expressed at E16, and transcript levels significantly changed in several areas during development.

479.9 DIFFERENTIAL EXPRESSION OF BRAIN L-TYPE AND P-TYPE CALCIUM CHANNEL mRNA IN ADULT AND DEVELOPING RAT BRAIN. H. Kim†, H.-L. Kim, and H. Chun. LMB, NINDS, NIH, Bethesda, MD 20892.

The four major types of voltage-sensitive Ca²⁺ channels present in the central nervous system (CNS) are classified as N-, L-, P-, and T-types. Earlier localization studies indicated that L-type Ca²⁺ channels are predominately expressed in those brain regions important for neurotransmitter function (Chapman et al., Trends Neurosci. 1989; 16:299-69, 1989). Several studies suggest that the P-type Ca²⁺ channels, initially identified in cerebellar Purkinje cells, are distributed more widely throughout the brain. Here, we investigated mRNA expression patterns of the α1 subunit of L- and P-type Ca²⁺ channels of adult and developing rat brains by in situ hybridization histochemistry, using the specific cRNA probes. The L-type α1 subunit transcript was abundantly expressed in the olfactory bulb, dentate gyrus, parietal and piriform cortices, superior colliculus, and facial nucleus; whereas the P-type α1 subunit was highly expressed in the hippocampus (CA3 region), geniculate bodies, inferior colliculus, and cerebellum. The cellular localization disclosed differential labeling of distinct cell types in various brain areas, suggesting that L- and P-type Ca²⁺ channels may be localized in specific subpopulations of neurons with this conclusion, relative L-type Ca²⁺ channel mRNA contents in different brain regions varied during development, whereas the P-type Ca²⁺ channel transcript gradually increased from very low levels during low levels during embryonic development (E16), peaked at the highest level at P12, and decreased thereafter in adults. The data reveal that spatial localization and temporal expression patterns of L- and P-type Ca²⁺ channel mRNA contents change developmentally, and suggest physiological role(s) for these channels in mammalian CNS ontogeny.


At least four different α1 subunit genes are expressed in the central nervous system, designated α₈ through α₄. We report the primary structure of the α₈ subunit and the functional expression of a human N-type voltage-dependent Ca²⁺ channel mediated by this α₁ subunit. Functional expression was achieved by transient coexpression of α₄ with human neuronal α₂ and β subunits [Williams et al. Neuron 87:1 (1992)] in mammalian cell culture. Whole cell recordings revealed a high-voltage activated, inactivating channel that was sensitive to blocking potential, insensitive to Bay K 8644 (1 μM) and irreversibly blocked by ω-conotoxin (0.5 μM) (see abstract by Feldman et al.) Transfected cells expressed a single class of high-affinity ω-conotoxin binding sites (Kₐ = 55 ± 18 pM; B_max = 25,000 ± 10,000 receptors per cell).

The predicted α₈ subunit structure consists of the characteristic twenty-four transmembrane topology domains characteristic of α₁ subunits. The predicted α₈ amino acid sequence is 64.1% and 43.0% identical to the rabbit Bi (α₂) dihydropyridine-sensitive and human α₁ dihydropyridine-sensitive subunits, respectively. α₈ has a characteristic large putative cytoplasmic loop between the IIßS and IIßII S transmembrane domains like the rabbit Bi subunit but is only 29.3% identical through a majority of the loop. The α₈ primary transcript is differentially processed to produce at least two isoforms, α₈a and α₈b, calculated molecular weights of 287,604 (40%) and 251,757 molecular weight (59%).
479.11

N-type Ca channels, found in neurons but not in muscle, are irreversibly blocked by omega toxin. Activation requires calcium-dependent depolarizations from negative holding potentials, and inactivation occurs over several minutes at 0 mV. We have now isolated cDNAs encoding a human omega toxin-sensitive (n-type) Ca channel and an n-type Ca channel with the properties of N-type channels.

Whole-cell recordings from transfected cells revealed Ba2+ currents up to several nA, irreversibly blocked by 0.5-10 μM omega toxin. At 0 mV, the current density was concentration dependent, consistent with rates measured in neurons. Activation began near -20 mV, and peaked near 10 mV. The current usually activated within 100 mV, and the time constant of rise of the peak was 60 ms (r0 = 1 s). Rates inward activation dominated at test pulses of 0 or 10 mV, r0 dominated at >10 mV. The currents were sensitive to holding potential, with a 20% decrease occurring at 40 to 60 mV and 90% inactivation near -40 mV. Currents were reversibly blocked by 50 μM Ca2+, and were not affected by dihydropyridines. Two observations suggest that the expressed channel is under tonic inhibition in the host cell; currents typically increased in magnitude 2 to 20-fold during the first several minutes of recording, and currents increased in magnitude when the test pulse was immediately preceded by a brief strong depolarization.

Thus, many of the properties attributed to N-type channels observed in neurons are exhibited by the recombinantly expressed channel.

479.12
CLONING OF A cDNA FOR A SYNAPTIC PLASMA MEMBRANE Na+/Ca2+ EXCHANGER: M. I. Michaelis*, J. Welch, K. Kumar, J. Foye and G. H. Davis, Depart. of Pharmacology and Toxicology, Univ. Kansas, Lawrence, KS 66045.
The Na+/Ca2+ exchanger is a plasma membrane protein which is believed to play a role in the regulation of Ca2+ fluxes, particularly in excitable cells. We have used a full-length cDNA encoding a human sodium-calcium exchanger (NCX subunit [see abstract by Ellis et al.] that, when transiently expressed in transfected mammalian cells with previously isolated cDNAs encoding human membrane Na+/K+ ATPase (Williamson et al., Neuron 8:711, 1992), expresses Ca channels with the properties of N-type channels.

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Thus, many of the properties attributed to N-type channels observed in neurons are exhibited by the recombinantly expressed channel.

480.1
AF64A (ETHYLCHELINE AZIDIRINUM ION) PRODUCES OXIDATIVE STRESS: RELATION TO CHLORITOXICITY AND FUNCTIONAL DEFICITS. J. Walsh, G. Weisbrod, R. W. Bacon, and E. Bondy*, Dept. Psychology, Rutgers Univ., New Brunswick, NJ 08903, and 1 Univ. of California, Irvine, CA 92717.

AF64A produces (1) a persistent decrease in pre synaptic cholinergic function, (2) a loss of cholinergic neurons in the medial septum, and (3) cognitive impairments. While AF64A inhibits high affinity choline uptake (HACHO) the mechanisms responsible for its long-term neurotoxicity are not well-characterized. The following experiments examined the potential role of oxidative stress in the cholinotoxicity and functional deficits induced by AF64A. Male Sprague-Dawley rats were bilaterally injected icv with artificial CSF or 3 mmol of AF64A. Rats were sacrificed two days following surgery and it was determined that AF64A increased the production of conjugated dienes, an index of lipid peroxidation and oxidative stress, and decreased the activity of choline CHAT in the hippocampus (HCPC).

Furthermore, there was a significant correlation between the degree of cholinergic toxicity and the extent of lipid peroxidation. In a subsequent study rats were p.o treated with saline or 50 mg/kg of the anti-oxidant Vitamin E (VE) ac 24 hrs and 15 min prior to icv injection of artificial CSF or AF64A (3 mmol/side). Vitamin E prevented the AF64A-induced (1) deficits in acquisition and retention in a Morris-water maze task and (2) the decrease in HACHO in the HPC. Thus, while the structure of AF64A leads to its accumulation in cholinergic neurons oxidative stress might significantly contribute to the cholinotoxicity and functional deficits induced by this compound. Supported by a Bush Grant to JTW.

480.3
EXCITOTOXIC LESIONS OF THE PEDUCULOPONTINE SEGMENTAL NUCLEUS IMPAIR RADIAL-ARM MAZE PERFORMANCE. Larry J. Butcher*, Justin D. Oh, and Cheryl Crase, Laboratory of Chemical Neuroanatomy and Dept. Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

Lesions of the pedunculopontine tegmental nucleus (PPT) lead to reduced choline acetyltransferase (ChAT) immunoreactivity in cholinergic basal forebrain nuclei (Oh et al., Soc. Neurosci. Abstr., 15, 782, 1989). In order to assess possible effects of PPT lesion on spatial memory, 10 female Sprague-Dawley rats with excitotoxic lesions were trained and tested on an 8-arm radial maze. After rats (n=5) reached a 90% error rate defined as no entries into an arm already entered, 3.8 mmol kainate (0.1 µl, total injection time = 4.5 min) was injected unilaterally into the PPT. Five additional rats served as controls and were injected with 0.1 µl of saline into the PPT. Starting on the day after surgery, and for an additional 6 days, rats were tested on the maze and the behavioral data was statistically analyzed with a 2-way mixed-design ANOVA. Lesioned rats, but not controls, showed a significantly greater impairment in spatial memory performance [F(1,12) = 12.143, Mse = 0.623, p<0.05]. These data suggest that excitotoxic lesions of the cells in the PPT nucleus have a deleterious effect on the spatial memory of rats.

[Support: USPHS grant NS 10928 to L.L.B.]

480.4
RADIAL-ARM MAZE PERFORMANCE IN RATS IS IMPAIRED BY VESAMICOL (N-TRANS-1,4-PHENYLDIPERIDINO) CYCLOHEXANOL, A POTENT INHIBITOR OF CHOLINE ACTIVE TRANSPORT BY SYNAPTIC VESICLES. Justin D. Oh*, Sonia Bockenauer, Antoine Keller, Stanley M. Parsons, Gary A. Rogers and Larry J. Butcher, Laboratory of Chemical Neuroanatomy and Dept. Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A. and Dept. of Chemistry, University of California, San Diego, CA 92093.

Effects on both choline acetyl-transferase (ChAT) immunohistochemistry and 8-arm radial maze performance were found for the drug Vesamicol, a potent inhibitor of acetylcholine (ACh) active transport by synaptic vesicles. Female rats (n=20) were randomly assigned either to vesamical group or vehicle control group. Rats received bilateral injections of 1 (ng vesamicol (2.0 x 0.5 mm) dissolved in PBS (pH=7.2) containing DMSO and acetic acid intraperitoneally in the nucleus basalis of Meynert (n=5) or intraventricularly (n=5). Rats injected with vesamicol showed significantly reduced numbers of CHAT-immunopositive cells in the basal forebrain 7 days following surgery and showed significantly increased the nucleus basalis of Meynert (n=5) or intraventricularly (n=5). Rats injected with vesamicol showed significantly reduced numbers of CHAT-immunopositive cells in the basal forebrain 7 days following surgery and showed significantly increased the nucleus basalis of Meynert (n=5) or intraventricularly (n=5). The nerve growth factor receptor revealed no actual cell loss or morphologic aberration in these cholinergic neurons. As measured by entries to repeat, radial-arm maze choice accuracy was significantly impaired in the vesamicol group compared to the control group (p<0.05). Paralleling the time-course of ChAT immunoreactivity decrease, the effects of vesamical on maze performance were significantly different from control group 6 and 7 days post-surgery. [Support: USPHS grant NS 10928 to L.L.B.].

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480.5

SELECTIVITY OF AN AFFINITY LIGAND, HEMICHLINOMUS MUSTARD, FOR THE HIGH AFFINITY CHOLINE TRANSPORT SYSTEM. K.H. Gylys* and D.J. Jenden Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

Hemicholinium mustard has been shown to be an irreversible inhibitor of high affinity choline uptake (HACU) in several preparations. (Smart, 1981, 1983; Gylys et al., 1990). In the present experiments the specificity of these irreversible effects was examined with respect to other cholinergic proteins and other sodium-dependent transport systems. To measure the effects of HCM on choline acetyltransferase (ChAT), synaptosomes were incubated in HCM, then washed. The synaptosomes were lysed and the ChAT activity was measured. Treatment with 50 μM HCM, a concentration that inhibits 100% of synaptosomal HACU, results in a 24% decrease in ChAT activity. In other experiments, rat brain membranes were incubated with 1 μM HCM and then washed before saturation studies with the muscarinic receptor ligand [3H]QNB were carried out. HCM pre-incubation showed no significant effect on either the affinity or number of muscarinic receptors. Dopamine (DA) transport is also relatively unaffected by HCM pre-treatment: 10 μM HCM, which inhibits HACU in synaptosomes by >90%, inhibits DA transport by 11%. These results support the use of HCM as an affinity ligand for HACU. (Supported by MH 17691)

480.7

CHOLINE BLOOD PRESSURE IN HYPOTENSIVE RATS IN Volvement of Vasopressin. V. Svec, I.I. Ulus*, S.Gürün, B.K.Kiran and L.R. Büyükuysal. Dept. of Pharmacology, Uludag Univ. Medical School., Bursa, TURKEY.

Left carotid artery of rats (280–350 g) was cannulated with PE 50 tubing to monitor blood pressure and animals were made hypotensive by bleeding (2 ml/100 g of body weight) or by treatment with histamine (10 mg/kg; ia), hekmasethionium (15 mg/kg; ip), phenylamine (5 mg/kg; ia) or 6-hydroxydopamine (50 mg/kg/iv). Intracerebroventricular (ICV) injection of choline (50–150 μg; rat) to these animals increased and restored blood pressure within 1–5 minutes after the choline treatment. Choline's effect was abolished by pretreatment of rats with necamylamine (50 μg). Pretreatment of rats with atropine (10 μg; ICV) failed to alter the effect of ICV choline. ICV choline (50 μg) also failed to increase and restore blood pressure when rats were pretreated with hemicholinium-3 (20 μg; ICV) 15 minutes before ICV choline. The increase in plasma levels of vasopressin was associated with the increase in blood pressure in ICV choline treated hypotensive rats. When animals were treated with an antagonist of vasopressin (10 μg/kg; ia) five minutes after ICV choline (150 μg) blood pressure decreased immediately to the pre-choline or to near pre-choline levels. These data indicate that ICV choline can increase and restore blood pressure in hypotensive rats by increasing the central cholinergic nicotinic neurotransmission. The increase in plasma levels of vasopressin involves in this effect of ICV choline.

480.8

EFFECTS OF APNEA ON BRAIN CHOLINE PRODUCTION IN RATS. O.U. Scremin* and D.J. Jenden. West L.A. V.A. Medical Center and UCLA School of Medicine, Dept. of Physiology and Pharmacology. Los Angeles CA 90024.

Free choline (Ch) concentration in brain tissue results from a balance between synthesis and hydrolysis of acetylcholine (ACh), synthesis and degradation of Ch containing phospholipids and exchange with plasma. Since the ability of brain tissue to synthesize Ch de novo is negligible, losses of this base through the circulation can have serious consequences for phospholipid and ACh metabolism. We tested the hypothesis that the energy deprivation associated with apnea would enhance the loss of Ch. Experiments were performed in rats, mechanically ventilated with a N2O/O2 mixture. Ch in plasma of aorta (Cha) minus that of retroglomoid vein (Ch v) multiplied by cerebral blood flow in the same vein represented the cerebral metabolic rate of Ch (CMRCh, mmol/min/kg) that reached -0.14±0.06 prior to apnea. Apnea of 1.5 min duration was followed by negative CMRCh values (-0.72±0.06 at 3 min post-apnea), returning to control after 16 min. Apnea of 3 min duration was followed by CMRCh values not different from zero during the initial 15 min followed by negative values (-0.52±0.11 at 60 min). Apnea of 6 min duration was followed by positive CMRCh initially (1.12±0.1 at 6 min) with negative values later (-0.74±0.23 at 64 min). Total Ch loss (mmol) during 1 hr was related to the duration of the apnea episode that preceded it (1.5 min= -12.05 ±3.57; 3 min= -17.09±6.13; 6 min= -27.32±4.15; Controls (no apnea)= -6.99±2.25. This enhanced Ch loss took place in spite of increased Cha. Peak Ch (mmol/ml) after apnea were: 1.5 min= 11.2±1.47; 3 min= 18.8±1.26; 6 min= 30.6± 1.07; Controls= 44.±2.1. It is concluded that apnea induces a significant loss of brain free Ch that could have metabolic consequences. Supported by the US Department of Veterans Affairs and USPHS MH 17691.
481.1  AUTORADIOGRAPHIC LOCALIZATION OF MUSCARINIC CHOLINERGIC RECEPTOR (MCR) SUBTYPES IN CAT BRAIN STEM. H.A. Baghdoyan*, M.T. Roth, R.B. Ducrow, and D.C. Magistretti. Dept. of Anesthesia, Penn State Coll. of Med., Hershey, PA, and Dept. of Neurology, Univ. of Miami Sch. of Med., Miami, FL 33136.

Brain stem cholinergic mechanisms are involved in generating REM sleep. Determination of specific MCR subtypes is not clear. The goal of the present study is to map the distribution of MCR subtypes likely to be important for sleep cycle control. Using the technique of Flynn et al. (Neurosci. Abst. 17; 586, 1991) which utilizes the kinetic properties of [3H]-methylscopolamine to differentially label MCR subtypes, we are localizing M1, M2, and M3 subtypes throughout the cat brain stem. Receptor density is quantified using CCD-camera-based image analysis. Preliminary results show that the feline medial pontine reticular formation (mPRF), a non-cholinergic, cholinergic region involved in REM sleep generation, contains very few M3 receptors (4.0 fmol/mg tissue). The pedunculopontine tegmental nucleus (PPT), which provides cholinergic input to the mPRF, also has few M3 receptors (7.4 fmol/mg). In contrast, the laterodorsal tegmental nucleus (LDT) has 18.8 fmol/mg of M3 receptors. Relatively high levels of M3 receptors were also localized to the periaqueductal gray (15.4 fmol/mg) and the substantia gelatinosa (22.0 fmol/mg). In the mPRF, LDT, and PPT, M1 and M2 receptors are being quantitated. Supported: Dept. of Anesthesia, HLA4749, MH45361 (HAB), NS24109 (RBD), NS27575 (DCM).

481.2  COMPARISON OF CHOLINERGIC PROPERTIES AND MUSCARINIC RECEPTOR SUBTYPES PRESENT IN TWO NEURAL CLONAL CELL LINES. M.A. BUCK, L.A. TAYLOR, V. REUPERT, C.E. TEDSSEND and R. McQUADE. CNS Pharmacology, Schering, Plough Research Institute, Bloomfield, NJ 07003.

Two cholinergic containing cell lines were investigated for various markers of cholinergic activity as well as characterized for muscarinic receptor subtypes. Muscarinic receptor characterization and identification in a neuronal cell line is of considerable interest to better understand cellular mechanisms responsible for the mode of interaction in the central nervous system. Cells of the homogeneous hybrid line of mouse mastocytoma X rat glioma (NG108-15) were compared with the PC12 phaeochromocytoma cell line derived from the rat adrenal medulla. PC12 and NG108-15 cells both demonstrated various levels of cholinergic activity. NG108-15 cells had the highest levels of M4 activity to choline acetyltransferase (CAT, 27.4 vs 13 nmol/mg protein) and acetylcholinesterase (AChE, 1216 vs 376 nmol/mg protein) than PC12 cells. Contrary, functional measurement of high affinity cholinergic (HACHT) indicated that PC12 cells contained almost double the activity. HACHT was sensitive to hemicholinium-3 (HC-3) and was modulated in both cell lines by muscarinic receptor agonists.

Muscarinic receptor characterization of the two cell lines was performed using saturation and competition binding studies with [3H]-acetylcholine (ACh) and characterized for the different affinity muscarinic receptor subtypes, m1-m5. Covalent labelling of the neuronal and transdifferentiated CHO cells with the muscarinic ligand, [3H]-propylenebicyclohexyl mustard (3H-PBBCM) and the mobilities of the mustard labelled species of these cells on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) confirmed the existence of different muscarinic cholinergic receptor subtypes in these cell lines.

481.3  ALKALOIDS ISOLATED FROM TROPICAL MARINE SPONGES BOUND TO MOLECULAR TARGETS IN RAT BRAIN MEMBRANES. G.E. de Motta, R. Rosa, A.D. Rodriguez, W. Silva and C. Jiménez*. Inst. of Marine Sciences and Department of Chemistry and Biology, U. of Puerto Rico and Dept. of Pharmacology, U. Central del Caribe.

Four structurally related C11-N1 compounds isolated from sponges of the genus Agelas exhibited both stimulatory and inhibitory activities in frog skeletal and smooth muscles. Potential target sites for these compounds were evaluated for their interaction with central cholinergic receptors (mAChR) and tetrodotoxin (TX)-sensitive sodium channels using rat brain synaptosomal membranes and radioligand assays. Competition experiments utilizing tritiated quinuclidinyl benzilate ([3H]-QNB) to label mAChRs revealed the following rank order of potency: sceptor > oxotremorin > dibromoscorpine > atropine. Competition experiments using tritiated saxitoxin ([3H-STX)] as a sodium channel marker yielded the following rank order of potency: sceptor > dibromoscorpine > oxotremorin > atropine. Scatchard analyses demonstrated that sceptor, the most potent member of this group, was a competitive inhibitor of both [3H]-QNB and [3H-STX] binding. (Supported by GM08102 and NS07464, NIH, and NOAA, UPR Sea Grant Program)


Methanolic extracts prepared from clonal cultures of Ostreopsis tenuisculus, a benthic dinoflagellate, were toxic to mice by i.p. injection. Reverse phase HPLC separation using isocratic methanol as the system solvent, produced two major fractions. Fraction I, with a retention time (R) of 2.04 min, contained apparently only one component while Fraction II consisted of a major component (R = 3.69 min) and several minor components. Using radioligand assay methods we studied the effect of the crude extracts and both HPLC fractions on muscarinic acetylcholine receptors (mACHR) in rat brain synaptosomal membranes. Preincubation of membranes with crude extracts (500 µg/ml) fraction I (500 µg/ml) and II (200 µg/ml) displaced binding of tritiated quinuclidinyl benzilate by 97.9± 3.7, 64.285± 3.5% and 61 ± 1.51%, respectively. In frog gastric muscle strips, a preparation we have shown to contain mAChR, crude extracts elicited contractions similar to those induced by acetylcholine in this muscle. (Supported by GM08102 and NS07464, NIH, and NOAA, UPR Sea Grant Program)

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481.7
HEXYLOXY-7TTP A POTENT AND SELECTIVE M1 ANTAGONIST 
INVITRO. C.H. Mich‡, F.P. Bymaster, D.O. Calligaro, S.J. Quimby, B.R. Sawyer, H.E. Shannon, J.S. Ward, P.H. Olsen, P. Sauerberg, M.J. Sheardown, P.D. Surdzik, Lilly Research Laboratories, Eli Lilly and Co., Ind., IN 46285 and Novo Nordisk, CNS Division, Mådøv, Denmark. Hexyloxy-7TTP (3-(4-hexyloxy-1,2,5-thiadiazole-3-yl)-1,2,5,6-
tetrahydro-1-methylpyridine) inhibited 3H-pirenzepine binding in rat hippocampus and 3H-oxtremorine-M binding in rat cortex with IC50 values of 7 nM and 10 nM, respectively. In 
denervated cells expressing human M1 receptors, hexyloxy-7TTP increased phosphoinositol turnover, and the potency and 
efficacy differed dependent upon the cell type in which the 
receptors were expressed (CHO-A9L>BHK). In denervated cells 
expressing m2, m3, m4 or m5 receptors, hexyloxy-7TTP was 
considerably less active. Hexyloxy-7TTP also was selective for 
M1 receptors in isolated tissues. At M1 receptors in rabbit 
vas deferens, it inhibited twitch height with an IC50 of 8 pM. 
Pirenzepine blocked the effects of hexyloxy-7TTP in rabbit 
vas deferens and had a Kb value of 10 nM. At M2 receptors in 
guinea pig atria, hexyloxy-7TTP had an IC50 of 50 nM. 
Hexyloxy-7TTP was a partial agonist in guinea pig ileum. In 
guinea pig bladder, it appeared as an agonist nor an 
antagonist. The present results demonstrate that hexyloxy-
7TTP is a potent, efficacious and selective M1 muscarinic 
agonist in-vitro.

481.8
EVIDENCE THAT M1 MUSCARINIC RECEPTOR SUBTYPE 
MEDIATE THE EFFECTS OF OXTREMORINE ON 
MUSCULOUS SEXUAL BEHAVIOR. S. Kesana and J. Velazquez-Moctezuma*, Dept. de Biologia de la 
Reproduccion, Universidad Autónoma Metropolitana Iztapalapa, 09340 Mexico D.F., Mexico. 
It has been shown that oxtremorine (Oxo), a muscarinic 
receptor agonist, has a facilitatory effect on muscular sexual behavior in rats. 
Muscarinic receptors have been divided in several subtypes. This study 
analyzes the possible participation of M1 muscarinic subtype in the 
mediation of OXO effects on muscular sexual behavior. Two 
groups of male rats received seven doses of the specific M1 antagonist 
(S)-atropine (6 mg/kg, i.p.) 30 min before assessing sexual behavior. 
Latency and frequency of mount, intromissions and ejaculations were recorded as well as 
inter-intromission interval. No changes in these parameters of 
sexual behavior were observed following TRl administration. 
In a different group of male rats, five doses of TRl were administered 
before OXO (0.4 mg/kg, i.p.). The facilitatory effect of OXO was 
completely prevented even with the smallest dose of TRl. These results 
strongly suggest the notion that cholinergic facilitation of muscular 
sexual behavior is mediated through the M1 muscarinic receptor 
subtype.

481.9
MODULATION OF ACETYLCHOLINE RELEASE 
BY MUSCARINIC RECEPTORS IS ALTERED FOLLOWING LESION 
OF CHOLINERGIC INPUTS WITH THE NEUROTOXIN AF64A. 
D. Thorne and P.E. Potter, Dept. Anesthesiology, Albert Einstein 
College of Medicine, Montefiore Medical Center, Bronx NY 10467. 
The effects of cholinergic agonists on the evoked release of 3H-
acetylcholine (ACh) were studied in male Sprague-Dawley rats in 
which hippocampal cholinergic terminals were lesioned with the 
neurotoxin AF64A (ethylcholine mustard aziridinium, 2 
rnles/ventricle). AF64A infusion, choline 
acetyltransferase activity was decreased by more than 40%. ACh 
release was evoked from superfused hippocampal slices by electrical 
stimulation (1 or 2 Hz, 2 min). The nicotine dose response curve was 
shifted to the left following treatment with AF64A. The EC50 
was shifted from 40 μM (controls) to 3.7 μM (AF64A). This change 
in response to nicotine was not due to changes in nicotinic binding sites. 
When the muscarinic receptors were blocked by atropine the nicotine 
dose response curve of control tissue, was shifted to the left (EC50=4.1 
μM). Atropine had no effect on the nicotine dose response curve of 
AF64A treated rats. In contrast, the dose response curve for 
inhibition of ACh release by the muscarinic agonist oxotremorine, 
was shifted to the right following AF64A treatment. This change in 
response to muscarinic drugs was reflected in a change in muscarinic 
M3 but not M1 binding sites.

481.10
LIGHT AND ELECTRON MICROSCOPIC LOCALIZATION OF m2 
MUSCARINIC RECEPTOR PROTEIN IN RAT SEPTUM AND 
HIPPOCAMPUS. A. Levy*, S. M. Hirsch and S. M. Edmunds, 
Dept. of Neurology, Emory University School of Medicine, Atlanta, GA 30322. 
Pharmacological studies indicate that muscarinic acetylcholine receptors 
preynaptically regulate the release of neurotransmitters. However, the 
precise identity of presynaptic genetic subtypes is unknown. 
The cellular and 
subcellular distribution of m2 receptor in medial septum and 
hippocampus was determined using subtype-specific antibodies (Levy et 
aI., 1991), avidin-biotin-peroxidase immunocytochemistry, and light 
and electron microscopic analysis. In medial septum, m2 immunoreactivity 
was present in both noncholinergic and cholinergic perikarya, and was 
more dense and punctate in the neurons. At the ultrastructural level, m2 
immunoreactivity was associated with the cytoplasmic face of the 
plasma membrane in perikarya, and in dendrites and postsynaptic densities: m2 
was also present in many terminals. In hippocampus, m2 was present 
in cell bodies and processes of large neurons in the stratum oriens and 
hilus. Neuripil immunoreactivity was abundant in the pyramidal neuron 
layer, the polymorph layer of dentate gyri. At the ultrastructural level, 
immunoreactivity primarily was in dendritic spines and rare axon 
terminals. Labeled spines contained reaction product within 
cytoplasm 
and within their postsynaptic densities. Contrary to current dogma, these 
findings indicate that in the septal region, m2 receptors are 
preynaptic (as well as postsynaptic), and that in hippocampus, m2 
muscarinic receptor immunoreactivity is postsynaptic. Supported by Alzheimer Association 
Faculty Scholar Award (AFL), NS 01387, and NS 30454.

481.11
MUSCARINIC RECEPTOR SUBTYPES INVOLVED IN CONTRACTION 
OF THE GUINEA PIG ILEUM. E.A. Thomas* and E.J. Lyle, 
Department of Pharmacology, College of Medicine, University of 
California, Irvine, CA 92717. 
Muscarinic receptor subtypes involved in smooth muscle contraction were 
investigated in the guinea pig ileum. The muscarinic agonist oxotremorine M (Oxo-M) 
caused concentration-dependent contractions of the isolated ileum (IC50=38 μM) 
with pharmacology consistent with that of an M3-mediated response. In 
order to characterize the M3 muscarinic receptors selectively, ilea were pre-treated with 
the irreversible M3 selective muscarinic agonist 4-DAMP mustard (N-(2-
chloroethyl)-4-piperidinyl diphenylacetate) (40 μM) for 1 h in the presence of the 
irreversible M1 antagontant AF64A (1 μM) and then washed extensively. 
This treatment caused a 22-fold rightward parallel shift in the concentration-effect 
curve for Oxo-M. In separate experiments, ilea were pre-treated with bistine (0.3 
μM) and then exposed (1 μM) before measuring oxotremorine-induced contractions. 
The resulting concentration-effect curve was biphasic consisting of high (50-500 μM) 
and low (>50 μM) affinity components. Similar treatment with 4-DAMP mustard and 
AF64A-116 only shifted the high affinity component, while producing a much 
egreater shift in the low affinity component of the curve. Treatment with AF64A-116 
(1 μM) abolished the high affinity component producing a monophasic 
concentration-effect curve. 4-DAMP mustard (10 μM; 1 h) also prevented 
M3- 
responsive muscarinic stimulation of phosphoinositol breakdown in the longitudinal 
muscle of the rat ileum, resulting in a 6.6-fold increase in the IC50 value with a 65% 
reduction of the maximal response. In contrast, this treatment only blocked 
M2- 
responsive mediated inhibition of adenylyl cyclase by a 2-fold increase in IC50, 
without affecting maximum inhibition. These results support the hypothesis that 
the M3 muscarinic receptors present in smooth muscle may influence contraction, 
perhaps by inhibiting relaxation induced by other receptors. Supported by N.I.H. 
Grant NS 35511.

481.12
THE (+) OPTICAL ISOMER OF (Z)-2-PCE SELECTIVELY BLOCKS 
M1, (ILEAL) BUT NOT M3, (CARDIAC) MUSCARINIC RECEPTORS. 
E.B. Thompson, M. Lu, S.M. Vogt and N.P. Ploughforth*, 
Departments of Pharmacodynamics, and Med. Chem. College of Pharmacy, 
and Pharmacology, College of Medicine, U.I. Chgo, IL 60612. 
Studies in our laboratory have utilized muscarinic antagonists as probes 
for exploring topographical areas of the muscarinic receptors. In 
these studies, direct clues as to the actual binding conformation of the 
hydrophobic portion of structurally flexible molecules (such as atropine and 
QNB) have been provided by use of synthetic analogues which are 
structurally locked into certain conformational states. (Lu et 
aI, 1991) showed that the racemic compound 2-phenyl cyclohexyl 
diethylamino ethyl ether ([Z]-2-PCE) was more potent in ileal (PA2 = 7.15) 
that in arterial (PA2 = 4.96) preparations. This suggests that the Z isomer 
is one of the most ideal selective muscarinic antagonist reported to date. In this 
study the optical isomers of the above compounds were synthesized and 
evaluated pharmacologically on isolated rat atria and ileum preparations. 
Ileal selectivity of the optical isomers was similar to that of the racemate. 
The (+) isomer was found to be a potent competitive antagonist by a factor 
>100 fold, for the ileal but not the atrial muscarinic receptors. Conversely, 
the (-) isomer appeared to be non-competitive in this ileum. The results 
indicate that the competitively antagonistic activity of the racemate is due to 
the (+) optical isomer.
481.13 PROPERTIES OF STRIATAL m4 MUSCARINIC RECEPTORS. S.L. Parkerton, H.B. Handeott, and L.T. Potter*. Molecular and Cellular Pharmacology, University of Miami School of Medicine, Miami, FL 33101. The rat striatum expresses an unusually high concentration of m4 muscarinic receptors, many m1 receptors, and a few m2 receptors (Levey et al. J. Neurosci. 11:2116). m1-Toxin quantitatively blocks the m1 receptors, and 95% of the residual receptors are m4 receptors (Parkerton et al. Neurosci. Abstrt 17:390). This approach permits the first binding studies of m4 receptors coupled to normal amounts of native G-proteins. The order of antagonist affinities was NMS, trihexyphenidyl, biperiden, oxotremorine, benzotropil, methoctramine, bethanechol, and aminopyrine, with aminopyrine, gallamine, and AF6116. Five of these ligands bound with the same affinity to rat striatal m4 receptors and human m4 receptors expressed in CHO-K1 cells. Agonist binding curves showed equal efficacies of Gpp(NH)p-sensitive high affinity (Kd) sites and Gpp(NH)p-insensitive low affinity (Kd) sites in both striatal and CHO-cell membranes. These studies suggest that m4, m2, m1 receptors (Potter et al. Mol Pharm 39:211), may be dimers. The order of Kd/Ka ratios (which often correlate with agonist efficacies) was oxo-M, oxotremorine, 4-acetylcholine, picrocarb, carbachol, methacholine and anesoline, and KA and Kd affinities were very similar in striatal and CHO cells. The order of Kd/Ka ratios, which often correlate with agonist efficacies, was oxo-M, carbachol, 4-acetylcholine, oxotremorine, anesoline and picrocarb, which would suggest that quaternary agonists work better than tertiary agonists. These data do not correlate well with the Kd values and efficacies found for agonists by McKinney et al. (Mol Pharm 40:1014) for the inhibition of cAMP levels in rat striatum. In their studies oxo-M showed high affinity and efficacy, but did so several tertiary agonists. Possible reasons for the different binding of the function data include effects of activating m1 receptors on cAMP levels, and the high activity of tertiary agonists on m2 receptors. Autoradiographic studies of m1 and m4 receptors confirm their wide distribution within the striatum, and indicate their distinct cellular locations.

481.14 ALLOSTERIC MODULATION OF MUSCARINIC RECEPTORS BY BASIC PROTEINS IN RAT CEREBRAL CORTEX AND HEART. J. He and E.E. El-Fakahany*. Division of Neuroscience Research in Psychiatry, University of Minnesota Medical School, Minneapolis, MN 55455. Allostere interactions of proteins, such as histones, myelin basic protein (MBP) and dynorphin A (1-13), with muscarinic receptors were investigated in rat cerebral cortex and heart using radioligand receptor binding assays. These basic proteins inhibited binding of the muscarinic ligand [3H]NMS at equilibrium and altered kinetics of its dissociation from the receptors. Histone VIII (rich in arginine) showed an inhibition constant value of 1.4 μM and a cooperativity value (n) of 4-5 in cerebral cortex. It also decreased the rate of dissociation of [3H]NMS with an IC50 of 0.9 μM and a maximal inhibition of 60%. No significant difference was observed between its effects in cerebral cortex and heart. MBP also inhibited [3H]NMS binding at equilibrium in a concentration-dependent manner with a lower n value (3.5 in cortex and 1.9 in heart). Maximal inhibition of specific [3H]NMS binding by MBP was reduced from 45% to 16% by increasing the concentration of [3H]NMS from 0.04 nM to 0.8 μM. The allosteric nature of MBP was also demonstrated in kinetic studies. The small basic peptide dynorphin A also exerted an allosteric effect on muscarinic receptor which was dependent on the number of basic residues. Our data suggest that positively charged amino acid residues in endogenous proteins might play a role in the regulation of the conformation of muscarinic receptors.

481.15 PHOSPHATIDYLCHOLINE-SPECIFIC PHOSPHOLIPASE D IS COUPLED TO RECEPTOR OCCUPANCY IN A9 CELLS TRANSFECTED WITH THE M3-MUSCARINIC RECEPTOR. P.G. Holbrook, J. Wendt and C.C. Felder, NIMH and NINDS, National Institute of Health, Bethesda, MD 20892. In the presence of ethanol, phospholipase D (PLD) catalyzes a transphosphatidylation reaction producing phosphatidyl-ethanol (PEI). PEI formed in A9 cells stably transfected with the rat m3 muscarinic coupled with carbachol (1mM) was isolated and analyzed by fast atom bombardment mass spectrometry. It had a molecular species profile identical to that of phosphatidylcholine (PC) from unstimulated cells. In cells labeled overnight in serum free media containing [3H]-palmitic acid and [3H]-PEI the stimulated formation of [3H]-PET required extracellular calcium (1mM) and was blocked by atropine (10 μM). TPA stimulated formation of [3H]-PET in the presence and in the absence of extracellular calcium. EC50 for carbachol stimulated [3H]-PET and [3H]-PA formation were respectively: 8.8 x 10^(-8) and 5.5 x 10^(-7) M. Experiments with receptor chimeras produced by switching the third cytoplasmic loops of the m3 and m2 receptors suggest that g-protein coupling is necessary for PLD-activation. Occupancy of muscarinic receptor subtypes that couple to PLC-specific PLC leads also to activation of a PC-specific PLC.

481.16 DIFFERENCES IN CARBACHOL BINDING AND PI TURNOVER BETWEEN THE DORSAL AND VENTRAL HIPPOCAMPUS. H. Ladinsky1, A. Garcia1, M. Zambelli1, G. Schiavi2, and S. Consolo2. Department of Biochemistry, Fondazione IRCCS Ospedale Maggiore Policlinico, Italy; Milan 20139 and Mario Negri Institute, Milan, Italy 20157. The full agonist CARB was more effective in stimulating PI turnover in the ventral (VH) than in the dorsal hippocampus (DH) while the partial agonist oxotremorine was similarly weak in both regions. No differences in density or distribution (>20% M1, 50% M3, 30% M4) of muscarinic receptor subtypes between DH and VH were found to explain the effect using several selective antagonists in competition experiments. From the affinities of pirenzepine (Kd 6.1 nM; Kd 757 nM) and DAU 6202 (4-hydroxy-3-(tropol oxycarbonyl-3,4-dihydroxy-1H quinazolin-2-one)RB 2.5 nM) to antagonize CARB-stimulated PI turnover, it appeared that about 50% of the response was activated by M1 and the other 50% by M3 receptors in either region. In binding studies against [3H]NMS, CARB recognized three agonist affinity states (SH, H, and L) in the VH and two (H and L) in the DH. Gpp(NH)p converted the SH and H to the L state in both regions. Thus, regional differences in types or concentrations of G proteins may account for the complexity with which CARB binds to muscarinic receptors and stimulates PI turnover in the areas.

481.18 COMPARISONS BETWEEN HUMAN MUSCARINIC RECEPTOR SUBTYPES COUPLED TO PHOSPHOLIPASE C AND THOSE COUPLED TO ADENYLATE CYCLASE: EFFECT OF RECEPTOR RESERVE. L.L. Lauffer, R.D. Schwarz, R.B., and C.J. Spencer. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105. Muscarinic receptors have been shown to exist as five distinct proteins which are functionally coupled to G proteins to phospholipase C (m1, m3, and m4) and adenyl cyclase (m2 and m5). Stable elevation of human receptors in CHO cells yielded cell lines with Ba2+ values ranging from 210-2450 fmoles/mg protein and Kd values of 0.1-0.8 nM (whole cell [3H]NMS binding). The use of these lines has allowed the pharmacological characterization of selectivities of various muscarinic agonists and antagonists to be determined using receptor binding and second messenger assays. Previous results obtained using PI hydrolysis, showed that both efficacy and potency of muscarinic agonists were markedly affected by receptor number, while antagonist results were not. However, it was not known whether the effects occurred in cyclase linked receptors. In the present study, activation of Hm2 and Hm4 receptors was performed in order to determine the effect of receptor number on agonist/antagonist-induced changes in cAMP formation. These results are compared to those obtained measuring PI turnover under similar conditions in Hm1, Hm3, and Hm5 cells.
842.1


Glutamate neurotoxicity is a major problem in the pathophysiology of numerous neurodegenerative diseases, including Stroke, Huntington's disease and Alzheimer's disease. Glutamate-induced neurotoxicity is mediated by the influx of calcium, however the biochemical pathways subsequent to calcium influx are poorly understood. Therefore, it would be useful to specifically alter calcium activated processes. Our laboratory has previously described a HSV-1 vector system which is capable of introducing genes into mature neurons (Science 1991;251:428-430). We are exploiting this vector system to genetically alter signal transduction pathways to elucidate intracellular mechanisms mediating glutamate neurotoxicity. Our initial studies characterized the ability of the HSV vector system to deliver genes into cortical neurons which have been previously used to assess glutamate neurotoxicity (Hartley and Choi, JPET 250:752, 1989). The prototype vector pHSVlac expresses E.coli B-galactosidase from the constitutive HSV IE 3/5 promoter. Following infection of these cortical cultures with pHSVlac, B-galactosidase expression was readily detected in 30-50% of the neurons, as well as in glia cells. Because of the importance of calcium in mediating glutamate neurotoxicity, we are expressing genes that affect calcium mediated processes and assessing their ability to alter glutamate mediated neurotoxicity.

842.2

DELAYED ADMINISTRATION OF MEMANTINE (MTE) PREVENTS NMDA RECEPTOR-MEDIATED NEURONAL DEATH. James W. Pollegrini*, M. Vincent Chen, Frances E. Jensen. Depted of Neurology, Children's Hospital and Progr. in Neurosci., Harvard Medical School, Boston, MA 02115.

Increasing evidence indicates that excitatory amino acids are responsible for neuronal cell death in a variety of neurological conditions including hypoxia-ischemia. The predominant form of neurotoxicity of the NMDA receptor-activated current may be mediated by the NMDA receptor. Recently, using rat retinal ganglion cell (RGC) cultures, we found that the anti-Parkinsonian drug MTE blocks NMDA-activated current by a mechanism of open-channel blockade ([IC50 = 1 μM]). Here, using single channel recording, we demonstrate that 12 μM MTE reduced the frequency of NMDA-elicited channel opening by 87%, shortened the mean open time by 30%, but did not change the unitary channel conductance. We had previously shown that MTE prevents NMDA receptor-mediated neurotoxicity when administered coincident with the insult in vitro or in vivo. We have now tested whether delayed administration of MTE can prevent neurotoxicity. Cultured RGCs were exposed to toxic (25 μM) glutamate levels in high Ca/low Mg medium and compared with control cells. By the next day, this insult resulted in neuronal survival of 35%. In sibling cultures, MTE (12 μM) was added at 0-7 h after glutamate exposure. Survival at 24 h was close to control levels when MTE had been administered at 0 or 1 h, and approached 80% after treatment at 4 h. Presently, we are using 7-10 day old Long-Evans rats in a bilateral carotid ligation stroke model. In 7/11 litter-matched pairs, animals treated 1 h after insult with MTE (20 mg/kg) had substantially smaller strokes by MRI and histopathology after 48 h.
482.5

A SHORT EPISODE OF SEIZURE ACTIVITY PROTECTS CA3 NEURONS FROM PROLONGED SEIZURE ACTIVITY-INDUCED DEATH.

I. Najeeb, S.S. Schreiber, A. Bruce, G. Tooco, and M. Baudry, Neurosciences Program, USC, Los Angeles, CA 90089-2520.

Systemic administration of kainic acid (KA) produces recurrent seizure activity and the loss of selectively vulnerable neuronal populations, in particular pyramidal CA3 neurons. We investigated the effects of a short episode of seizure activity on CA3 neuronal death following subsequent prolonged seizure activity. One group of adult rats was subjected to 1 hr of KA-induced seizure activity which was terminated by pentobarbital anesthesia, and treated again 16 hrs later with KA. Another group received KA twice at 16 hrs interval, while a third group received only one injection of KA at 16 hrs. No KA administration. Animals which were subjected to a short seizure episode exhibited a marked protection against delayed neuronal death in CA3 neurons resulting from a subsequent KA-induced episode of seizure activity. Histological examination of the brains of animals sacrificed 5 days after the last KA administration indicated a total absence of neuronal loss in CA3 as compared to animals which had been treated with one or two KA injections and which showed extensive neuronal loss. Immunocytochemistry of heat shock protein 72 (HSP72) performed 16 hrs after a short episode of seizure activity indicated that this period was sufficient to cause an increase of HSP72 in CA3 pyramidal cells suggesting that HSP72 could participate in the neuronal protection observed under these conditions. This phenomenon of “seizure tolerance” might therefore be viewed as an analog of the phenomenon of “ischemic tolerance” described in the brain.

(Supported by NIH grants NS 01337 to SSS and NS 18427 to MB).

482.7

SELECTIVE ANTAGONISM OF JORO SPIDER TOXIN (JSTX) AGAINST QUISQUALATE (QUIS)-INDUCED LESION IN THE HIPPOCAMPUS.


QUIS, a non-NMDA agonist, is known to provoke seizures with a selective cell loss in the hippocampus, indicating a useful neurotoxin for a specific experimental model of epilepsy. We reported that a JSTX analogue, 1-naphthylacetylspermine (1-NA-Spm), exerts a potent and selective suppression of hippocampal epileptic discharges induced by QUIS (Brain Res., in press).

The present study was conducted to evaluate whether 1-NA-Spm selectively antagonized against histological changes induced by QUIS as well. Male Wistar rats were injected icv with 1-NA-Spm followed by either QUIS or quisqualate (QUIN), a NMDA agonist. After behavioral changes were observed, the animals were subjected to histological examination. QUIS (30μg) resulted in the loss of CA3 pyramidal cells in the injection side, which was completely blocked by 1-NA-Spm (50 μg) pretreatment. Contra-lateral side remained intact irrespective of the history of repetitive seizures. JSTX analogue alone (80 μg) had virtually no effect on the cellular architecture in the hippocampus. QUIN (30 μg) induced CA1/4 lesion which was refractory to JSTX pretreatment. In several rats, JSTX appeared to exacerbate QUIN-induced changes.

482.8

LOW POTENCY OF COMPETITIVE NMDA ANTAGONISTS AGAINST GLUTAMATE NEUROTOXICITY DUE TO ASTROCYTE UPTAKE.


A puzzling feature of the pharmacology of glutamate neurotoxicity is the low potency of competitive NMDA antagonists compared to what would be expected on the basis of radioligand binding studies. These experiments were performed in order to examine the role of glutamate uptake in determining the apparent pharmacology of competitive antagonists in tissue culture models of glutamate neurotoxicity.

The potencies of APV against the transported agonist glutamate and the non-transported agonist NMDA in astrocyte-rich and astrocyte-poor cortical cultures were determined. Both antagonists were used at approximately 5 times their EC50. The IC50 of APV against glutamate (1mM) in astrocyte-rich cultures was approximately 1500 μM compared to 25 μM against NMDA (200 μM). The difference in potency disappeared in astrocyte-poor cultures in which the IC50 for APV against both glutamate (20 μM) and NMDA (200 μM) was 60-70 μM.

These results are best explained by a model in which glutamate uptake plays an important role, and in which toxicity at the dendrite and at the cell body can be differentiated.

482.9

PICOLINIC ACID MODULATES KAINIC ACID INDUCED GLUTamate RELEASE FROM RAT STRIATAL SLICES.

L.C. Vrooan, M. Pacaud, D. Desaix, and B. Bading. Department of Pharmacology and Toxicology, Queen’s University, Kingston, Ontario, Canada, K7L 3N6.

Picolinic acid (PICA), a pyridine monocarboxylic acid and metabolite of tryptophan, protects neurons from excitotoxic damage that is dependent on the presence of intact glutamergic input. It is hypothesized that PICA may produce its effect by inhibiting glutamate (Glu) release. In the present study, we examined the effect of PICA on KA-induced Glu release (1 μM) induced Glu release from rat striatal slices. Endogenous Glu release was analyzed by HPLC with fluorescence detection. A dose response relationship was observed for the inhibition of the KA-induced Glu release by PICA. PICA (100 μM) was found to significantly inhibit KA-induced Glu release by 65%, an effect similar to that produced by the selective non-NMDA receptor antagonist DRCX (500 μM). PIC inhibited the calcium dependent component of releasable Glu. Nicotinic acid and icotinic acid, two structural analogues of PIC, showed similar profiles. PICA alone was found to significantly increase basal release of Glu by 44%. These results suggest that PIC has a stimulatory as well as an inhibitory action on striatal Glu release.

(Supported by the Medical Research Council of Canada)
482.11

AMANTADINE INHIBITS EXCITOTOXICITY IN CEREBROSPINAL FLUIDS
H.S. Lustig, K.J. von Braunschwich & J. Chen and D.A. Greeneberg,
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Excitatory amino acids (EAAs) have been implicated in the pathogenesis of acute and chronic neurodegenerative processes, and
EAAs antagonists are potent against excitotoxicity in a variety of in vivo and in vitro disease models. Certain antiparkinsonian
drugs, including amantadine, inhibit EAA responses mediated through N-
methyL-D-aspartate (NMDA)-gating EAA receptors and compete for
(P)MK-801 binding sites on NMDA receptor-gated ion channels.
Therefore, such drugs might not only reduce parkinsonian symptoms, but also modify neurodegeneration. Using neuron-enriched cultures
from embryonic rat cerebral cortex, we investigated the effect of amantadine on NMDA-induced toxicity, determined by lactate
dehydrogenase (LDH) release, and on NMDA-stimulated elevation of intracellular Ca2+ (Ca2+). Measured by fluorescence video imaging
with fura-2, LDH release from control cultures (25±2% of total LDH) was unaffected by amantadine alone at concentrations as high as
300 μM (231%). LDH release, measured 24 hr after exposure for 20 min to 100 μM NMDA, was increased to 46±3%. Pretreatment for 10 min with amantadine (10-1000 μM) inhibited the toxicity of 100 μM NMDA, with half-maximal inhibition at 30 μM and complete inhibition at 300 μM. Amantadine (100 μM) also reduced the rise in
Ca2+ produced by NMDA. These findings indicate that amantadine and other antiparkinsonian drugs with NMDA receptor antagonist
properties may protect against excitotoxic neuronal injury.

482.12


7-Chlorokyurenic acid (7-C1-KYNA), a selective antagonist of the glycine site associated with the NMDA receptor, is neuroprotective in experimental test systems. Since the compound penetrates poorly into the brain, it is difficult to explore its therapeutic potential for excitotoxic brain diseases. We have now examined if 4-chloro-
kyurenic (4-C1-KYNA), which should have easier access to the brain, can serve as a bioprecursor of 7-C1-KYNA. Cerebral cortex
extracts, incubated with [ring-13C]alanine and NADPH-diaphorase, were prepared from rat and human brain. Enzymatic production of the latter
was confirmed in all cases. In kinetic experiments with rat KAT, 4-C1-KYNA behaved like an effective competitive inhibitor, thus further
indicating its ability to serve as a substrate of KAT. Preliminary evidence suggests that conversion of 4-C1-KYNA to 7-C1-KYNA can also take place in the rat brain in vivo. Since (rat) brain KAT is predominantly localized in astrocytes which are often seen in
position to exocytotic edema, 4-C1-KYNA utilization may provide a means to produce 7-C1-KYNA in astrocytes in anatomicallly
distinct and pharmacologically relevant foci. Supported by grants NS 16102 and NS 28236 (to RS).

482.13

EXCITOTOXIC HIPPOCAMPAL DAMAGE AND NEUROPROTECTIVE AGENTS.

Glutamate agonists, such as quisqualate, kainate, N-methyl-D-aspartate (NMDA), quisqualate, and AMPA were injected intracerebroventricularly in rats to induce convulsive reactions and hippocampal damage in order to model glutamate-mediated brain injury. Animals showed typical convulsive effects and heavy lesions of hippocampal
region. In rats treated systemically with non-competitive glutamate agonists magnesium sulfate, MK 801, ketamine, or the adamanate derivative nembutal, a reduction in neuropathological signs was observed. Depen-
ding on the dose and the application schedule even complete protection was observed. Factors of the glutamate transmitter mechanism were found to be affected in lesioned areas, most prominent the activity of aspartate
aminotransferase. The vulnerability of neurons containing nitric oxide synthase (NADPH-diaphoresis stained) was observed to be related to the type of glutamate agonist used as a noxious stimulus.

482.14

REPEATED EXPOSURE TO GLUTAMATE ALTERS BOTH ITS POTENCY AND EFFICACY AS AN EXCITOTOXIN. J.M. Dubinsky.
Department of Physiology, University of Texas Health Science Center, San Antonio, Texas 78284-7756.

Transient increases in extracellular glutamate (GLU), associated with ischemic events in vivo, are thought to contribute to the eventual neuronal loss observed several days later. One hypothesis explaining this delayed excitotoxicity states that normal synaptic release of GLU becomes toxic following the initial ischemic event. To test this hypothesis, in vitro toxicity experiments were
performed on cultured hippocampal neurons experiencing two successive GLU insults. In the first toxic exposure, all cultures received 5 min of mild toxic concentrations of GLU. Dose-response curves for the percentage of surviving neurons were constructed for varying doses of GLU applied during the second 5
min exposure, 2 hr after the first. Control dose-response curves following simple solution changes as a first exposure were
characterized by an EC50 of 0.162 μM. GLU was used as the first exposure, the EC50 for the second exposure dropped slightly to 49 μM. When 100μM GLU was applied during the first exposure, the EC50 decreased to less than 5 μM. However, the amount of survival in both experiments was greater than expected from the control dose-response curve. The increase in both efficacy and potency indicate complex interactions may be involved in understanding GLU toxicity following an ischemic event. This work was supported by NIH #AG10034.
**482.17**

AGE DEPENDENCY OF NMDA ANTAGONIST NEUROTOXICITY
Barber, MT, Price, J, Luhovyy, TA Fuller* and JW Olney, Washington Univ., St. Louis MO 63110.

Antagonists of the NMDA subtype of glutamate receptor, including phencyclidine (PCP), MK-801 and ketamine, protect neurons against excitotoxic injury in conditions such as ischemia, hypoglycemia and status epilepticus. However, in rats these agents modulate neurotoxic side effects (pathomorphological changes in cerebrocortical neurons), and in humans, both PCP and ketamine are known to cause acute schizophrenia-like psychotic symptoms. The neurotoxic reaction associated with ketamine anesthesia, known as an "emergence" reaction, is suppressed by certain drugs (barbiturates and benzodiazepines) that also block the neurotoxic reaction in rat cortex, suggesting a relationship between the neurotoxic and psychotomimetic actions of these agents. The ketamine "emergence" reaction, like schizophrenia, is peculiarly age-dependent—typically, susceptibility is greatest in early to mid adulthood, and pediatric populations are not vulnerable. Here we report that susceptibility to the cerebrocortical neurotoxic reaction is also age-dependent with rats being non-susceptible to MK-801 neurotoxicity at 1 month of age, weakly susceptible at 2 months and not fully susceptible until 3 months.

Thus, a similar age-dependency profile characterizes: 1) the neurotoxicity of NMDA antagonists in rats, 2) the psychotomimetic effects of NMDA antagonists in humans, and 3) schizophrenia psychosis, and susceptibility to other adult-onset neurological disorders. Our findings suggest the possibility that some or all of these phenomena may be mechanistically related, and that NMDA antagonists may be safer to use as anesthetics in infancy than in adulthood. Supported by DA 06454, DA05072, AG 05681 and RSA MH 38894 (JWO).

**482.19**

CALPAIN I INHIBITION DOES NOT BLOCK EXCITATORY AMINO ACID-INDUCED NEUROTOXICITY IN MURINE CORTICAL CULTURES. V.M. Brown and R.G. Giftard, Dept. of Anesthesia, Stanford University, Sch. of Med., Stanford, CA 94305.

Activation of the Ca2+-dependent protease calpain I has been implicated in neuronal responses to excitatory amino acids in such diverse settings as long term potentiation and ischemic brain damage. Spectrin breakdown subsequent to Calpain I activation by increased intracellular calcium has been related to neuronal death, but reports of its involvement in the development of excitatory amino acid neurotoxicity have varied in different systems. We investigated the effect of Calpain I inhibitors in cortical cultures injured by exposure to maximal and submaximal concentrations of the glutamatergic excitatory amino acids (NMDA), kainate and AMPA. Injury was assessed by release of lactate dehydrogenase. We studied both calpain inhibitor I and MDL 28170 which has high specificity for calpain II, both up to 100μM. Neither inhibitor protected neurons from brief exposure to NMDA (100μM-500μM) or 24 hr exposure to NMDA (12.5μM-500μM), kainate (20μM-300μM) or AMPA (10μM-100μM). MDL 28170 has been shown to inhibit Ca2+ ionophore A23187-induced proteolysis of spectrin in erythrocyte ghosts. We then investigated the effect of the drug on A23187-induced toxicity in our cultures. Cells exposed to the ionophore for 20 minutes were moderately injured when assessed at 24 hr. MDL 28170 reduced the neuronal damage by about 30%. Activation of calpain I does not appear to be a major source of injury in excitatory amino acid-induced neurotoxicity in neocortical cultures. Sponsored in part by NS 01425.

**482.20**

L-trans-2,4-pyrrolidine dicarboxylic acid (L-PDC), an Inhibitor of High Affinity Glutamate Uptake (HAGU), is Neuotopic in Neonatal Rat Brain. John E. Barber* and Faye S. Silverstein, University of Michigan, Departments of Pediatrics and Neurology, Ann Arbor, MI 48109.

Strong evidence of the neurototoxicity of endogenous glutamate (GLU) in mammalian brain was provided by the observation that DL-threo-3-hydroxyaspartate (THA), an HAGU inhibitor, was neurotoxic in the dorsal rhomboid striatum ([Neurochem 44:247]), however, THA did not elicit neuropathologic changes in the developing brain. The absence of injury was interpreted as evidence that immaturity of gluatamatergic innervation limited the potential toxicity of endogenous GLU at this developmental stage. Yet, the specific mechanism for the hypothesis that endogenous GLU can be neurotoxic in the developing brain. To address this issue, we assessed the neurotoxicity of the selective HAGU inhibitor L-PDC ([Med Chem 34:717]) in 7 day old rats. L-PDC (75 μM) was injected into right anterior striatum (STR) (568 nmol, n=2) or through dorsal hippocampus into posterior STR (568 nmol, n=4, 150 nmol, n=2). Neuropathology was assessed in animals killed 5 days later. After anterior injections, focal neuronal necrosis was evident in dorsal STR. High-dose posterior injections caused prominent hippocampal lesions with CA1.3 pyramidal layer thinning and focal necroses in dorsal thalamus. Small foci of pyramidal cell loss. Focal cortical necroses and caudal cysts were apparent adjacent to the injection track. Preliminary autoradiographic 3H-GLU binding assays were also done; L-PDC (10 μM or 50 μM) did not displace 3H-GLU, suggesting that L-PDC lacked intrinsic agonist properties. Thus, L-PDC-induced brain injury provides direct support for the hypothesis that endogenous GLU may be neurotoxic in the developing brain.

**EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY III**

**483.1**

DISCOVERY OF A HIGH AFFINITY, SODIUM-DEPENDENT L-PROLINE TRANSPORTER EXPRESSED IN SUBPOPULATIONS OF PUTATIVE GLUTAMERGIC NEURONS.

We have used PGP with degenerate oligonucleotides derived from two conserved regions of the norepinephrine and GABA transporters to identify novel sodium-dependent carrier in brain. One PCR product hybridized to a 4 kb RNA concentrated in subpopulations of putative glutamatergic neurons. Prominent in this localization signals were observed over cortical cells of the olfactory bulb, pyramidal cells of layer V of the cerebral cortex, pyramidal cells of the piriform cortex, and pyramidal cells of field CA3 of the hippocampus. In contrast, background labeling was observed over granule cells of the dentate gyrus, caudate-putamen, white matter tracts, facial plexus, and ependymal cells of the cerebral ventricles. Transient expression of the gluta-1 cDNA constructs in sodium-dependent, high affinity (Km=9.7 μM) L-proline uptake in Hela cells which exhibited a pharmacological profile similar to that for high affinity L-proline transport in rat hypothalamus. The cloned transporter CTPD predicts a 637 amino acid protein with 12 putative transmembrane domains and exhibits 44-45% amino acid sequence identity with other members of the emerging family of neurotransmitter-sodiumdependent syperactive findings to high affinity L-proline in specific excitatory pathways in the CNS and provide the basis for a direct molecular analysis of the presynaptic components of these excitatory projections.

**483.2**

CHANGES OF pH DURING GLUTAMATE UPTAKE. M. Bonier*, M. Szatkowska, A. Amato and D. Archbishop, Dept of Physiology, University College London, Gower St, London WC1H 0BT, UK.

Uptake of glutamate into glial cells and neurons ultimately terminates the post-synaptic action of the neurotransmitter. The glutamate uptake carrier is known to be powered by the co-transport of an excess of Na+ ions (at least 2) and it also counter-transportes one K+ ion.

When L-glutamate or D-aspartate uptake was activated in whole-cell clamped Müller cells from the salamander, a pH sensitive electrode outside the cell detected an alkalization of the extracellular medium, and intracellular BCECF detected an internal acidification. Intracellular pH was not perturbed when cells were perfused with CI−, outside when stimulated with CI− inside the cell. These data suggest that a pH changing anion such as OH− or HCO3− is transported out of the cell by the uptake carrier and that CI− can be transported in place of this anion. Inhibition of carbonic anhydrase by acetazolamide had no effect on the intracellular acidification or the extracellular alkalization, suggesting that HCO3− is not counter-transported on the carrier.

We therefore propose an uptake stoichiometry in which one glutamate ion is transported into the cell together with 2 Na+ ions, and one K+ ion and one OH− are counter-transported, per cycle of the anion (Supported by the Wellcome Trust, M.R.C., and Science Plan of the European Community)

The glutamate-gated, linked mGluR1 and the ion-channel-complex of the NMDA receptor mediate slow and fast responses of neurons to glutamate, respectively. We find that both mGluR1 and NMDA receptors are widely distributed. However, in a number of brain regions, they occur in distinctly different neuronal populations.

For instance, mGluR1 predominates in the non-pyramidal neurons of the cerebral cortex, stratum oriens of CA1 and CA3 of the hippocampus, islands of Calleja, subthalamic nucleus, lateral hypothalamic area, and the ventricular layer of the cerebellum. In contrast, the NMDA receptor predominates in the pyramidal neurons of the cerebral cortex and the hippocampus, medium spiny neurons of the striatum, paraventricular nucleus of hypothalamus, and the granule cell layer of the cerebellum.

These findings, in combination with reports of the distribution of other glutamate receptors, suggest that while many neurons are equipped to mediate both slow and fast responses to glutamate, many others are specialized to have either a slow or fast response.

483.4 DISTRIBUTION AND MORPHOLOGY OF NEURONS EXPRESSING THE GLUR 2/3 SUBUNIT IN FOUR NEOCORTICAL AREAS OF MONKEYS. Francesco Conti* and Andrea Minelli. Institute of Human Physiology, University of Padua, Via Ranieri, 160031 Ascona (Italy).

In the last few years, several non-NMDA ionotropic glutamate (Glu) receptor subunits (Glur) have been cloned. Four of these, Glur 1-4 (or Glu A-D), have pharmacological properties of AMPA receptors and appear to form heterometamer receptor complexes. Recent data show that the Glur 2 subunit appears to be dominant in determining the properties of the receptor complex, since its presence is required for the "C"-type activity. Here we report the distribution and morphology of neurons expressing the Glur 2/3 subunit in four neocortical areas of the monkey brain as studied using a subunit-specific antibody produced and characterized in Dr. Wentholt's lab (Wentholt et al., J. Biol. Chem., 267: 501-507, 1992). Under deep barbiturate anaesthesia, animals were perfused transcardially with phosphate buffer followed by 4% paraformaldehyde, the brain was removed, and postfixed. Small blocks from the primary somatic sensory (areas 3a, 3b, 1, and 2), the primary visual (area 17), the primary motor (area 4) and the posterior parietal (area 5) cortices were cut on a Vibratome in 15-µm thick sections, and processed for Glur-ICC (Ab 25) using the ABC method. Immunostaining was present both on cell bodies (excluding the nucleus) and on the major dendrites, and in the neuropil (mostly on dendritic processes).The pattern of Glur 2/3-positive neurons was basically similar in the four areas studied and was characterized by the presence of numerous small and medium sized pyramidal neurons in layer II and upper layer III, sparse non-pyramidal neurons in layer lower III and in layer IV, and some pyramidal and non-pyramidal neurons in layers V-VI. Some differences were, however, present when comparing the four areas: the number of positive neurons in layer IV was highest in areas 17 and 5, whereas the number of pyramidal neurons in layers V-VI was highest in area 4. The identity of immunoneactive non-pyramidal neurons could not be determined at the light microscope, and experiments are in progress to define the ultrastructural features of these neurons.

483.5 EVIDENCE FOR GLUTAMATERGIC NONPYRAMIDAL NEURONS IN THE RAT HIPPOCAMPUS: GOLGI STAINING COMBINED WITH POSTEMBEDDING IMMUNOCYTOCHEMISTRY. E. Soriguera*, H.-D. Hoffmann* and M. Frotscher*, †-Unit of Cell Biology, Faculty of Biology, Univ. Barcelona, Spain 08028; †-Inst. Anat., Univ. Freiburg, D-7800 Freiburg, FRG.

Nonpyramidal neurons of the hippocampus are known to be inhibitory using GABA as a neurotransmitter. They control the principal cells, granule cells and pyramidal neurons, which are excitatory. By using postembedding immunocytochemistry in combination with Golgi staining, we provide evidence here that at least two types of nonpyramidal neurons in the rat hippocampus use glutamate as transmitter.

Many cells in the hilus and a novel type of spiny nonpyramidal cell in the mossy fiber projection zone of CA3 were Golgi-stained, gold-toned, and processed for postembedding immunocytochemistry using glutamate and GABA antibodies. At the electron microscopic level, both cell types were seen to receive many inputs from the mossy fibers. When adjacent sections through the cell body region of Golgi-stained neurons were immunostained, both cell types reacted for glutamate, like the pyramidal and granule cells, but did not stain for GABA. In contrast, many other local circuit neurons were GABA-positive but could not be immunostained with the glutamate antibody. Exclusory glutamatergic nonpyramidal cells that are integrated in the main excitatory pathway of the hippocampal circuit may play a hitherto underestimated role in hippocampal circuitry. (Supported by the DFG: Fr 620/1-5 and FIS 991262-2)

483.6 PHOSPHATE-ACTIVATED GLUTAMINASE mRNA EXPRESSION IN RAT SPINAL CORD. B. Styrivasaas* and K.E. Miller. Dept. Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Phosphate-activated glutaminase (PAG) is the enzyme responsible for converting glutamine to glutamate in glutaamatergic and GABAergic neurons. We have examined PAG mRNA in rat spinal cord using RNA blots and in situ hybridization. Spinal cords, kidneys, and brains from normal rats and total RNA was extracted, and slot blots were prepared. 32P-labelled PAG cDNA (ATCC: BamH I, EcoRI) was used to probe RNA blots. PAG mRNA was expressed in spinal cord similar to brain and kidney. To localize PAG mRNA to neurons, in situ hybridization was performed on sections from cervical, thoracic, and lumbar spinal cord and dorsal root ganglia (DRG). 32P-labelled PAG cDNA was used for autoradiographic localization and digoxigenin-labelled PAG cDNA or 20mer PAG oligoprobes were used for nonradioactive localization. In the DRG, most neurons were labelled. In addition to intensely labelled motor neurons, other labelled spinal neurons were found in the intermediolateral cell column, lateral spinal and cervical nuclei, marginal zone, substantia gelatinosa, and central gray region (lamina X). The results from this study indicate that PAG mRNA is expressed in specific neurons of rat DRG and spinal cord, including some neurons not previously considered glutamatergic or GABAergic. Supported by NS27213 (KEM).

483.7 THREE SUBPOPULATIONS OF CORTICAL GABAERGIC INTRINSIC NEURONS IN THE RAT. Y. Kubota* and P.G. Jones Laboratory for Neural Systems, Frontier Research Program, RIKEN, Wako, Saitama 351-01, Japan and Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Recent studies have revealed that the cortical GABAergic neurons contain a wide variety of other neuroactive substances and specific calcium binding proteins. In this study we demonstrated the subpopulations of GABAergic neurons in different cortical area of the rat with double immunohistochemical methods. In all areas of the seocortex, intrinsic GABAergic neurons are subdivided into at least three types based on the selective colocalization of parvalbumin, cabindin D 28kd or choline acetyltransferase (ChAT). All cabindin D 28 kd/GABA immunoreactive cells show somatostatin immunoreactivity and a small number of cabindin D 28kd containing cells also show neuropeptide Y immunoreactivity and NADPH-diaphorase staining. All ChAT/GABA immunoreactive cells also show VIP immunoreactivity. In the piriform cortex and entorhinal cortex, these three subpopulations of neurons have slightly different characteristics. GABAergic parvalbumin containing neurons always contain cabindin D 28kd but about half the cabindin D 28kd containing neurons do not show parvalbumin immunoreactivity.

483.8 GLUTAMATE, GABA AND SUBSTANCE P IN THE PARAVENTRICULAR NUCLEUS OF THE THALAMUS (PV): AN IMMUNOELECTRON MICROSCOPIC STUDY IN THE RAT. G. Balercia, S. De Biasi*, A. Ambrosi* and M. Benussi. University of Verona. Italy and Department of General Physiology and Biochemistry, University of Milan, Italy.

PV is part of the midline group of thalamic nuclei and its synaptic organization and neurochemical features are still largely unknown. Immunopositivity for GABA and glutamate (Glu) in the rat PV was here revealed by a post-embedding immunogold procedure, and that for substance P(SP) by a pre-embedding method, using polyclonal antiser. Glu, GABA, SP-immunopositive (IR) terminals were observed whereas only Glu-immunopositivity was also detected in neuronal perikarya, thus confirming the extrinsic origin of the GABAergic and Spergic innervation of the rat PV. Glu-IR terminals contain round clear vesicles and occasionally large granular ones; they make asymmetric synaptic contacts with proximal and distal dendritic arborizations. GABA-immunolabeling is in small to medium-sized terminals that contain clear pleomorphic vesicles and make symmetric synapses on dendrites of different caliber and frequently on cell bodies; some of them also contain several large granular ones. Sp-IR arborizations are present in small, dome-shaped terminals contacting distal dendrites, and in medium- to large-sized terminals contacting proximal dendrites and their spines. SP-IR axon terminals containing clear and contain round clear vesicles and a variable number of large granular vesicles. Supported by NIH NS27872 and Italian CNR and MURST.
483.9

GLUTAMATE-IMMUNOREACTIVE CLIMBING FIBERS IN THE RAT CEREBELLAR GLAIR CORTEX. P. G. Glende, F. C. Oertig, L. Hennequet, J. Gondra and P. Stress. Dept. of Neuroscience, Basque Country University, E-48080 Bilbao; 1Insttitut für Hirnforschung, University of Zürich, CH-8029 Zürich.

The nature of the climbing fiber transmitter is still a matter of debate. To determine whether glutamate-immunoreactive profiles with neurobiological characteristics of climbing fibers could be found in the rat cerebellar cortex, an immunocytochemical study was performed at the levels of light (LM) and electron microscopy (EM). A monoclonal 'anti-glutamate' antibody was used in combination with postembbeding staining. At the LM level, strongly labeled fibrous profiles and chains of interconnected varicosities appeared, often in close apposition to principal Purkinje cell dendrites. In EM preparations, some immunoreactive presynaptic elements were varicosities and elongated profiles that were 3-4 times more heavily labeled than their post-synaptic partners. These results show that a subset of climbing fibers and their terminals contain relatively high levels of glutamate-like immunoreactivity and provide additional evidence for a role of glutamate as transmitter in these cerebellar afferents. Supported by Proyecto del Gobierno Vasco grant 9108, DGICYT grant PB91-0087 and Swiss National Foundation grant 31-27822-89.

483.11


As part of a broader investigation we tested whether terminals of large fibers mediating low-threshold cutaneous input are enriched in glutamate or aspartate. Four to 7 days after injection of HRP conjugated to B subunit of cholera toxin in the sciatic a., rats were perfused with 2.5% glutaraldehyde/0.5% paraformaldehyde/0.1% picric acid. Vibration sections of lumbar spinal segments were cut, reacted for TMB and embedded in plastic. Blocks from lamina I were cut out; thin sections were stained with glutamate and aspartate antisera separately or in combination using different sizes of gold particles. Quantitative EM methods were employed to compare the density of gold particles coding for either antiserum in labeled terminals. To help define background, sections were also stained for GABA.

Mean staining level for glutamate was above background in primary afferent terminals in lamina IV compared with staining in dendrites, glia and GABA-positive terminals. Staining for aspartate was only marginally higher than background in afferent terminals and was elevated in dendrites as well. Density of staining for both amino acids in primary afferent terminals in lamina IV was lower than that in terminals of primary (unmyelinated and small myelinated) fibers in lamina II (Phenax et al., Neurosci. Abst.1991).

483.12


We have previously observed GLU neurons and processes in the ventrolateral subnucleus of the tractus solitarius (VNTS) using light microscopy. In this study, we have attempted to define the types of immunoreactive profiles which are found in the VNTS. Adult mongrel cats were anesthetized and perfused through the descending aorta with a combination of 4% paraformaldehyde and 0.2% glutaraldehyde. Vibratome sections were processed by an avidin-biotin based immunoperoxidase method utilizing a mouse anti-glutamate antisera (IMSTAR). We have observed several small GLU perikarya with a large invaginated nucleus and a thin rim of immunoreactive cytoplasm. In addition, we have seen a few large GLU neurons with similar characteristics. Numerous large GLU dendrites receiving primarily asymmetric synapses from unlabelled nerve terminals were also seen. GLU axons were observed and unmyelinated. As yet, we have not observed any GLU nerve terminals in the VNTS. These data suggest that some of the inspiratory neurons which predominate in the DRG utilize glutamate as an excitatory neurotransmitter, however, as yet the data do not indicate a role for glutamatergic nerve terminals in the regulation of the activity of DRG neurons. Supported by NIH HL 1RO1-44822 and the American Heart Association.

483.13

GLUTAMATE AND ASPARTEATE IMMUNOREACTIVITY IN CORtical STROTRAL NEURONS OF THE RAT - B. Glutamata* - Ist. Fisiologia umana, University of Catania, I-95125, Catania ITALY.

The aim of the present work was to test whether cortical neurons are immunostained by antisera raised in rabbits against glutamate and aspartate conjugated to hemocyanin by glutaraldehyde (Hepler et al., J. Histochem. Cytochem., 1988, 36: 13-22). Corticostriatal neurons were identified by the retrograde transport of a tracer (colloidal gold-labeled WGA-apoHRP; Basbaum and Menétrey, J. Comp. Neurol., 1987, 261: 306-318) pressure-injected in the caudate-putamen; antisera were visualized by peroxidase immunocytochemistry using the ABC method. The bulk of corticostriatal neurons was observed in layer V of frontal and parietal areas, mainly in the ipsilateral hemisphere. In a sample of 1019 corticostriatal neurons in sections processed for Glu-antiserum, 595 (or 59%) were immunostained; in a sample of 1470 corticostriatal neurons in sections processed for Asp-antiserum, 837 (or 57%) were immunostained. Since it has been previously demonstrated that the two antisera largely reveal distinct populations of cortical neurons, results obtained combining Glu- and Asp-immunocytochemistry indicate that the entire population of corticostriatal neurons contains elevated concentrations of Glu or Asp in their cell body.

483.14


The nucleus raphe pallidus (NRP) has been described to evoke, upon electrical stimulation, a rapid depolarization and action potential discharges in cat hindlimb motoneurons of both flexor and extensor origins (Fung and Barnes, 1989, Neuroscience, 20: 183-190). We sought to uncover two fundamental questions pertaining to such findings: (1) whether the motor excitation is caused by rapheospinal neurons or other descending systems traversing through or adjacent to the medullary sites of stimulation; (2) is the fast, excitatory glutamatergic (Glu) neurotransmitter involved in the bulbospinal excitation of hindlimb motoneurons?

Using the simultaneous, dual (Glu and serotonin) immunofluorescence method in combination with retrogradetract-tracing technique (by unilaterally injecting FITC- labeled microspheres to L7 ventral horn), spatially projecting neurons arising from NRP and nucleus raphe obscurus (NRO) were frequently found to be found in L7 of either or both anterograde, with more triple-labeled compared to double-labeled cells. Electrical stimuli (4 pulses of 50 μA duration, 50 10-15 μA stimulation, 90-190 μs) applied to these medullary sites, in decerebrate cats, potentiated the L7 monosynaptic reflex (MSR). That the NRO or NRP neurons (instead of adjacent fiber systems) were the main source of the MSR enhancement was confirmed by a simultaneous recordings of the MSR upon chemically stimulating these rapheospinal neurons with 0.2-0.5 μl of 5.0 M glutamate. Microinjections of the vehicle to the same sites with glutamate showed no changes on the ventral root responses. These data support a role of glutamate in rapheospinally induced excitation of lumbar cord somatomotor outflow in cats.

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483.15


Glutamate (Glu) is considered to be a major excitatory neurotransmit-
ter in the central nervous system. Previously reported presence of Glu-like immunoreactivity (Glu-IR) is not observed in the rodent catalcholaminergic nocu-
locus coeruleus (LC) raises two questions: (1) Are there any Glu-IR neurons in the catalcholaminergic/clopisynetic system of the cat? (2) What is the physiologi-
ical role, if any, of Glu in LC control of spinal motoneurons?

In this study we used simultaneous immunofluorescence technique to iden-
tify tyrosine hydroxylase (TH) and Glu immunoreactivities in neurons that were retrogradely labeled by viral (L7 ventral horn) injections of
flourescent microspheres (Zhou et al., 1992, J. Chem. Neuroanat. 5:1-10). We found a widespread association between Glu and TH in most (>80%) de-
sorbing neurons and they were distributed randomly throughout the
nocrocardial extent of the LC complex. Electrical stimulation (4 pulses of 50
an d d t 1 00 Hz, intensity = 50-200 uA) of the LC complex, in
decerebrate cats, consistently induced L7 motoneuron discharges recordable
(isually as ventrol root responses. The LC evoked ventral root re-
sponses were reversely reduced (>50%) upon intraperitoneal injections of
the non-NMDA antagonist CNQX (in a total of 4 pere trial along the vent-
ral cone, each penetration consisted of 2 injections of 5 ng/0.2 uL).

Vehicle injections were without any significant effect on the LC evoked
ventral root responses. These results suggest that Glu has a pivotal role in
mediating coeruleosympathetic excitation of lumbar motoneurons in cats.

484.1 DISTRIBUTION OF THE NMDA RECEPTOR ANTAGONISTS [3H]C-
GCS 19755 AND [3H]MK-801 AFTER INTRACRANIAL INJECTION

The tissue distribution of [3H]C-GCS 19755 and [3H]MK-801 was in-
vestigated for up to 6 h after single lumbar spinal i.t. injection. [3H]C-
GCS 19755 distributed slowly from its injection site towards
brainstem and cortex. In the cortex, the radioactivity peaking 3-4 h after
injection. At no time did the level in frontal cortex exceed 10
% of the level in lumbar spinal cord. The highest peripheral level of
[3H]C-GCS 19755 was found in kidneys. [3H]MK-801 redistributed
rapidly from the spinal cord injection site to peripheral organs. The
highest peripheral levels of [3H]MK-801 were found in lungs and liver,
where the radioactivity peaked between 10 and 30 min after the
injection. The levels of [3H]C-GCS 19755 were consistently higher in
CNS tissues (except for the first 15 min in frontal cortex) and bleed
the corresponding levels of [3H]MK-801. The opposite relationship was
true in liver, lungs, kidneys, stomach, intestine, spleen and heart.

The effect of the latency in the hot plate test was quantified in the same animals immediately prior to sacrifice for
the distribution study. For the first hour after the injection, the
effect of CGS 19755 in the hot plate followed the temporal
distribution of this antagonist to the lumbar region of spinal cord.
MK-801 was substantially less effective in the hot plate test even at 100
times the effective equimolar dose of [3H]C-GCS 19755. The masked
difference in the distribution profile of [3H]MK-801 and [3H]C-GCS
19755 in the spinal cord does not appear to explain the lack of effect of
MK-801 in the hot plate test.

484.2 EFFECTS OF GLYCINE ON DIZOCYLIDINE-INDUCED CHANGES IN
ACOUSTIC STARTLE. R.S. Mambach. Dept. of Pharmacology &
Toxicology, Medical College of Virginia, Richmond, VA 23298-0619.

Dizocilpine significantly elevated startle magnitude and eliminated
prepulse inhibition, but glycine (17 and 56 mg/kg) and milcarnine (10-100 mg/kg)
had no effect. Moreover, administration of these compounds did not affect
changes in response magnitude and pre-pulse inhibition produced by
dizocilpine. A higher dose of glycine, 170 mg/kg, had no effect on startle
magnitude but in combination with dizocilpine killed 42% of the subjects
tested. Except for the lethal effects of glycine-dizocilpine combinations,
these preliminary results do not indicate that behavioral effects of NMDA
antagonists acting at the PCP receptor are modified by exogenous glycine.
Further work with glycine antagonists and selective agonists at the
strypnine-insensitive glycine binding site will explore other possible
interactions with PCP-like drugs. Supported by PHS grant MH-46631.

484.3 NOVEL ANALOGS OF DEXTROMETHORPHAN ANTAGONIZE NMDA

Dextromethorphan (DM) and its major metabolite
dextrorphan (DX) possess significant
anticonvulsant activity. We have previously reported on the anticonvulsant effects of a novel series of 3-substituted DM analogs to protect rats
against maximal electroshock-induced convulsions. To date, three analogs have been synthesized which are more potent anticonvulsants than DM. In
this study we explored the potential anticonvulsant nature of these DM analogs in a rat model of NMDA
(15 nM, i.c.v.) convulsions. Pretreatment with DM (20-80 μg, i.c.v.) delayed the onset to NMDA-
induced "popcorn" convulsions, whereas having little
or no effect on the incidence of convulsions. DX (2.5-
20 μg, i.c.v.) attenuated both latency and
incidence. Of the three analogs tested, the
ethyether derivative of DM, AHN0136 (20-80 μg,
mc), was the most potent anticonvulsant
exhibiting complete protection against NMDA
cconvulsions. The isopropyl ether and primary amine derivative were at least as potent as DM. These
results will be discussed relative to
receptor mechanism of action, behavioral
side effects and possible clinical usefulness.

484.4 CONANTOKIN PEPTIDE FROM CONUS GEOGRAPHUS
MODULATES [3H]NMDA-PERIDINE (TCP) BINDING TO THE
NMDA CHANNEL AND REDUCES NMDA RECEPTOR CURRENT.
L. L. Couplingour, D. M. Rock, C. Harchin, J. Hawley and G. W.
Campbell*, Parke-Davis Pharmaceutical Res. Div., Warner-Lambert
Co., Ann Arbor, Ml 48106-1047.

C. conatus (CT-G) is a seventeen amino-acid peptide extracted from the venom of the marine snail Conus geographicus. It previously
has been shown to interact with the NMDA subtype of glutamate
receptor. In well washed Triton-treated rat brain membranes the
binding of [3H]TCP was markedly enhanced upon the addition of
the NMDA channel agonist, glutamate and glycine. The maximal
enhancement caused by glycine (no added glutamate) was reduced in the presence of CT-G (2 εM). The maximal enhancement by glutamate
(no added glycine) was unaffected, but the concentration-response curve for glutamate enhancement was shifted to the right by CT-G. In
addition, CT-G did not affect the binding of [3H]AMPA or [3H]kainate
to non-NMDA receptors. This data suggests a selective effect on the
glycine site of the NMDA channel.

In cultured rat cortical neurons CT-G (2-20 εM) reduced the
amplitude of whole-cell NMDA receptor currents and the frequency of
NMDA-evoked single-channel openings in excited outside-out patch
clamp recordings. Inset of the reduction of whole-cell single-channel current by CT-G was slow, and the reductions were not readily
reversible.

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THURSDAY AM
484.5 KINETIC ANALYSIS OF NMDA MODULATORS ON [H]MK-801 BINDING. D.P. Carrozzi*, K. Williams and P.B. Molinoff, Dept. of Pharmacol., Univ. of Penn., Phila., PA 19104.

The NMDA receptor complex contains a distinct binding site for polyamines, including spermine, which enhance the binding of [H]MK-801, at a site within the ion channel. In the present studies, analysis of the kinetics of association of [H]MK-801 was used to investigate potential mechanisms of action of polyamines. Experiments were performed to determine whether specific modulators alter the conformation or dynamics of the receptor, which might be detected as an increase in the observed association rate (K_a). The observed association rates were biphasic in the presence of glutamate and spermine (g) or g plus spermine and were not altered by preincubation of membranes with these modulators before addition of [H]MK-801. This suggests a mechanism that involves a change in the duration of the open and closed states rather than a persistent change in the conformation of the receptor. In other experiments, the rates of association (fast and slow) were independent of the concentration of g plus spermine (30 μM), a condition which results in maximal changes in equilibrium binding. These results, which were an unexpected finding, suggest that the limiting factor of [H]MK-801 binding even under these conditions, involves access of ligand to its binding site. This phenomenon may involve changes in the dynamics of channel opening initiated by activation of the polyamine site (supported by USPHS grant GM47481 and NS30000).

484.6 DIAMINES INHIBIT NMDA RECEPTOR RESPONSES THROUGH A CHANNEL-BLOCKING MECHANISM. S. Subramaniam*, S.D. Donevan and M.A. Rogawski, Epilepsy Research Branch, NINDS, NIH, Bethesda, MD 20892.

It has been proposed that the inhibitory actions of the diamines diaminodiacetate (DA-10) and diaminodiacetate (DA-12) on NMDA-receptor responses is due to their actions as inverse agonists at the polyamine site. Recently, we and others have observed that the polyamines spermine and spermidine can block the NMDA receptor through an open channel mechanism. In this study we examined whether diamines could affect NMDA channels through a similar mechanism. In whole-cell recordings from cultured rat hippocampal neurons (V_m = -60 mV), the diamines inhibited NMDA currents in a concentration-dependent fashion. The efficacies of the diamines increased with increasing length of the carbon chain. Thus, DA-12, the longest diamine tested, was the most potent (IC_50 = 7 μM), whereas diaminopropene (DA-3), the shortest diamine tested, produced only partial inhibition (~70% at 10 mM). The blocking action of the polycationic diamines decreased at positive holding potentials supporting a channel blocking mechanism. In outside-out patches, DA-12 produced a flicker block of NMDA-induced single-channel currents whereas the shorter diamine putrescine (DA-4) produced an apparent reduction in channel amplitude. The ability to resolve flickering with DA-12 but not with the less potent shorter chain diamine DA-4 may reflect differences in binding kinetics but we cannot rule out the possibility of a distinct mechanism of block for the shorter diamines. These observations highlight the potential for using diamines as blockers of NMDA currents through an open channel mechanism, and therefore it may not be necessary to invoke inverse-agonist properties of these compounds to explain their inhibitory actions on NMDA receptor responses.

484.7 NEUROCHEMICAL TOLERANCE TO MK-801 (DIZOCILPINE) IN THE MOUSE. P.H. Hutson, I.J. Bristow, K. Flaitman, L. Thorn and M.D. Trickelburg, Merck Sharp and Dohme Research Laboratories, Neurosciences Research Centre, Terlings Park, Harlow, Essex CM3 2QQ, U.K.

In addition to anticonvulsant and neuroprotective effects, acute administration of the non-competitive NMDA receptor antagonist, MK-801 (dizocilpine) stimulates the turnover of dopamine in corticostriatal brain. We now show that chronic treatment with MK-801 decreases the neurochemical response to the compound in the nucleus accumbens, but not in the medial prefrontal cortex (mPFC).

Dihydroxyphenylacetic acid (DOPAC) was determined in mouse brain regions by HPLC and electrochemical detection. In mice killed 60 min after drug administration (15 mg/kg, s.c.) to minimize the concentration of DOPAC (mg/g tissue) by 60 and 85% in mouse nucleus accumbens and medial prefrontal cortex respectively (Table 1). Fatty eight hours after i.p. injection of 10 mg/kg MK-801, this same dose given 60 min before death again markedly increased DOPAC concentration in our medial prefrontal cortex (89%), but to a much reduced response in the nucleus accumbens (18%). Neither acute nor chronic drug treatment altered striatal DOPAC concentration.

Table 1: Chronic saline Chronic MK-801

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<th>N. Accumbens</th>
<th>saline</th>
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Thus, mesolimbic and mesocortical dopaminergic neurons appear to be differentially affected by chronic treatment with MK-801.

484.8 IFENPRODIL SELECTIVELY POTENTIATES NMDA-INDUCED DEPOLARIZATIONS IN THE RAT CORTICAL WEDGE IN VITRO. P.L. Herring* and D.A. Lowe, Sandox Research Institute, P.O. Box,CH 3006 Bern, Switzerland.

Ifenprodil, an agent thought to interact with the polyamine site of the NMDA receptor (Cartier et al. Europ. J. Pharmacol. 164:611, 1989), was studied in cortical wedges as described by Lowe et al.(Neuro.Letters 113:315, 1990). Excitatory amino acids (EAAs) were added to ACSF perfused into the chamber containing the cortical layers. Effects were quantified by determining the area under the curve of EAA-induced depolarizations. In Mg2+-free ACSF Ifenprodil (10 to 1000μM) potentiated NMDA-induced depolarizations by 50 to 187% over controls (mean±SD: 97±52). This potentiating effect was not observed in ACSF containing 1mM [Mg2+] and was attenuated at 0.2 mM [Mg2+]. The potentiation was fully reversed by 5μM 7-chloro-kynurenic acid (7CI-KYAC). If the potentiation was established before 7CI-KYAC application it reapplied after washout of the antagonist. Depolarizations elicited by non-NMDA EAA agonists such as AMPA and quisqualate were not affected by Ifenprodil. The potentiating effect of Ifenprodil is therefore NMDA specific and the experiments with Mg2+ and 7CI-KYAC suggest a site of action deep within the NMDA operated channel.


Polyamines are known to modulate excitatory synaptic transmission mediated by N-methyl-D-aspartate (NMDA) receptor. We tested the effect of increased putrescine levels in transgenic mice line that overexpresses human ornithine decarboxylase on the spatial learning/memory and the epileptic seizure threshold. Concentrations of putrescine in the different brain areas of transgenic mice increased from undetectable (limit 5 μmol/g) to more than 60 μmol/g compared with their nontransgenic littermates. Contents of spermidine and spermine remained unchanged. Transgenic mice had impaired appetitive water maze learning test (expressed using task latency). Transgenic mice also increased seizure threshold to clonic phase of pentyleneetetrazol-induced seizures and to tonic phase of corneal electroshock convulsions. The effect against electroshock convulsions was potentiated when putrescine content was further increased in the levels of 5 μmol/g. These results suggest that putrescine may play an important role in the excitatory neurotransmission.

484.10 EFFICACY OF SYNTHETIC POLYAMINES IN MODULATING NMDA-MEDIATED INCREASES IN INTRACELLULAR CALCIUM. J.M. Fahy*, G.L. Pitchard, L.G. Miller. Dept. of Pharmacology and Experimental Therapeutics and Neuroscience Program, Tufts Univ. School of Medicine Boston, MA 02111.

The endogenous polyamines spermine and spermidine potentiates N-methyl-D-aspartate (NMDA) receptor ionophore-mediated events. We have previously shown that both spermine and spermidine are able to potentiate NMDA-mediated increases in intracellular calcium ([Ca2+]i) of cultured chick cortical neurons using the calcium sensitive fluorescent dye Fura2. The present study characteristics structurally related synthetic polyamines. IBPA (3,3’ imino-bis-propylene) and AEPD (N-(2-aminoethyl)-1,3 propanediamine) are triamines which differ from spermidine in the length of one aliphatic chain between central terminal amines. IBPA potentiated NMDA-mediated increases of [Ca2+]i at concentrations of 100 , 250, 500 and 1000 μM. Its effects at these concentrations were also approximately 40% greater than effects of spermine. AEPD produced an increase of [Ca2+]i only at1 mM. Diaminodiacetate(DA10) is a putative inverse agonist at the NMDA receptor ionophore and in this system decreases NMDA-mediated increases of [Ca2+]i. No other diamine tested(DAS-DAE) had a similar effect. Only DAS(cadaverine) at 1 mM significantly altered NMDA-mediated responses by increasing, not decreasing, [Ca2+]i. As previously reported, the effect of putrescine may predict the functional efficacy of a synthetic polyamine but it is strongly influenced by the properties of other sidechains.

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NMDA antagonists, such as MK-801, block induction of hippocampal long term potentiation (LTP) and impair the performance of rats on various behavioral tasks, especially acquisition of spatial learning/memory tasks. NMDA antagonists also produce neurotoxic side effects. Two hours after subcutaneous injection of a single low dose of MK-801, pathomorphological changes appeared in the hippocampal cortices. A neurochemical definitional receptor is implicated in this neuropathic reaction in that the reaction is blocked by certain antimuscarinic drugs, including scopolamine. Since this neurotoxic reaction occurs in the hippocampal cortices and not hippocampal neurons, the question arises whether the memory impairing effects of MK-801 might reflect a functional disruption by high sinapical neurons. In this case, scopolamine might block the memory-impairing action just as it blocks the neurotoxic action of MK-801 on cortical neurons. Using a hole board test and cross-over design, we found that MK-801 (0.05 mg/kg, i.p.) compared to scopolamine (0.025 mg/kg ip) or saline, significantly impaired the ability of male rats to learn a novel location after 4 hours a food reward. However, when scopolamine (0.025 mg/kg ip) was coadministered with MK-801 (0.05 mg/kg ip), rats performed like saline controls and better (marginally nonsignificant, p = .077) than MK-801-treated rats. Moreover, significantly more animals were unable to learn the task under the influence of MK-801 alone (7/23) compared to MK-801 + scopolamine (0/23). These findings suggest, contrary to popular assumption, that the locus of action of NMDA antagonists in causing a performance deficit in learning tasks may be the cortical rather than the hippocampal. If so, it may be necessary to reinterpret prior behavioral studies in which performance deficits in learning tests have been attributed either explicitly or implicitly to an action of the drug on LTP-like mechanisms. Supported by R01 MH 38894 (JWO), DA 05072, DA 06454 and AG 10561.

484.12 INTRACRANIAL INJECTION OF Dopamine IN A DENERVATED STRIATUM REVERSES CIRCLING INDUCED BY THE NMDA ANTAGONIST MK-801. J.A.St-Pierre, L.Giguere and P.J.Bédard, Centre de Recherche en Neurobiologie, Hôpital Ste-Justine, Laval University, Quebec City, Canada.

Cats were treated with 6-OHDA in the left medial forebrain bundle. Fifteen days later, they were tested with amphetamine (0.25mg/kg s.c.). Microinjections of different doses of dopamine (1, 25 or 50µg/µl) were performed by guide-cannula stereotactically into the striatum. Dopamine induced a contralateral circling response, in a dose-dependent manner, which was projected into the lesioned side. Similarly, microinjection of dopamine into the lesioned side followed by stereotactic injection of MK-801 (100 µg/µl) 20 minutes later, produced a contralateral circling response. However, this circling response persisted with 4 fold that of the circling induced by dopamine alone. As previously reported in unilateral lesioned rats, MK-801 alone, produce an ipsilateral circling response (Co-Pierre et al., Soc for Neurosci, 1991) whereas microinjection of dopamine combined with MK-801 reverses the circling elicited. Taken together, our results suggest that the potentiation seen in the present experiment cannot be explained by increased release of dopamine. In addition, the circling response induced by the suppression of the glutamatergic neurotransmission seems to be facilitated by dopamine receptor activation. Supported by MRC of Canada.


Three ionotropic receptors (NMDA, KA and AMPA) which bind L-glutamate are present in various CNS structures. However, their existence on mesocorticlimbic dopamine (DA) neurons is less clear. Information about the factors controlling the excitability of these neurons may provide some insight into the neurobiology of schizophrenia, and the reinforcing properties of drugs of abuse. In midbrain slices extracellular recordings were made from VTA DA neurons identified by location, pacemaker-like firing, a rate of < 5 spikes/s, and action potential durations > 2 ms. Superfusion with NMDA, KA and AMPA produced dose-dependent reversible excitations at all 52 neurons tested. The threshold for NMDA (N=17) activation was 1.3 µM, with 10 and 30 µM increasing rates by 1.7 and 3.0 spikes/s. The threshold for KA (N=25) was 300 nM-1 µM, with increases of 1.5 and 2.4 spikes/s occurring at 10 and 100 µM. AMPA (N=10) was the most potent excitant, with a threshold at 100 nM and increases of 3.4 spikes/s at 1 µM. Also, none of the agonists changed the firing pattern from regular to bunting activity. The effects of NMDA were antagonized by 30 µM CGS 19755, while KA and AMPA were selectively blocked by 10 and 1 µM NBQX. The antagonists did not affect spontaneous firing. These data indicate that the excitability of VTA neurons is under the influence of the three glutamate receptor subtypes with a rank order of potency of AMPA > kainate > NMDA.


Intracellular and extracellular recordings were obtained from SPN in neonate rat spinal cord slices. Focal electrical stimulation of the dorsal horn or lateral funiculus evoked an EPSP which was both voltage-dependent and potential-sustained over several action potentials. The EPSP was partly reduced by the selective NMDA receptor antagonists APV, CPP and MK801 and was completely abolished by the non-NMDA receptor antagonist CNQX. Superfusion of the selective EAA receptor agonists AMPA, kainate, quisqualate, NMDA and ACDF induced concentration-dependent depolarisations in all neurons tested. AMPA, kainate and NMDA-induced responses were blocked by a cocktail of both NMDA (APV, CPP or MK801) and non-NMDA (CNQX or DOPQ) antagonists, whereas responses to quisqualate and ACDF persisted. Depolarising responses to quisqualate and ACDF were associated with an increase in neuronal input resistance and decreased in amplitude with increased membrane hyperpolarisation. In a subpopulation of cells, quisqualate and ACDF induced oscillations in membrane potential which gave rise to rhythmic burst firing at higher agonist doses.

We conclude that both AMPA and NMDA subtypes of receptors mediate synaptic transmission in SPN. Glutamate can also act via a metabotropic receptor to excite SPN by reducing a potassium conductance, and in some SPN to induce rhythmic activity.

This work is supported by the British Heart Foundation, the Wellcome Trust and the MRC.

484.16 KYNURENIC ACID MODULATES EXCITATORY AMINO ACID-INDUCED EXCITATION OF DOPAMINE-CONTAINING NEURONS IN RAT SUBSTANTIA NIGRA. H.-Q. Wu, R. Schewe and P.D. Shepherd, Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kyurenic acid (KYNA), an endogenous antagonist of ionotropic excitatory amino acid (EAA) receptors, was tested for its ability to modulate NMDA and AMPA-induced excitation of dopamine (DA)-containing neurons in the rat substantia nigra (SNc). Experiments were conducted using conventional extracellular recording techniques in conjunction with a simple data preparation. Bath application of NMDA (1-20 µM) or AMPA (1-10 µM) produced a concentration-dependent increase in the firing rate of SNc DA neurons. The highest concentration of both agonists produced a rapid and reversible cessation of activity that was attributed to acute induction of depolarization block. KYNA (10 µM - 1 mM) inhibited the excitatory effects of NMDA in a concentration-dependent fashion (DC50 = 100 µM) without affecting basal firing rate. KYNA (100 µM) proved slightly more potent in inhibiting the effects of AMPA. Perfusion of tissue slices with low Mg2+ Ringer's (0.12mM) increased the NMDA-induced excitation of DA neurons but failed to affect the ability of KYNA to antagonize the effects of NMDA. Addition of glucose (up to 100 µM) or kynurenic acid (1 mM) to the perfusion medium of KYNA, to the bathing solution had no effect on either basal firing rate or the increase in firing produced by concomitant application of NMDA. Co-application of D-serine (100 µM) partially attenuated the ability of KYNA to block the excitatory effects of NMDA. Thus, the ability of KYNA to block NMDA-induced excitation of mesolimbic DA neurons may involve an interaction with the glycine allosteric site on the NMDA receptor. Taken together, these data suggest that endogenous KYNA may play an important role in regulating DA cell excitability by modulating EAA neurotransmission.
EXCITATORY AMINO ACIDS: RECPTORS VI

485.1
GLUTAMATE AND GLYCINE ACT SYNERGISTICALLY TO STIMULATE "[H]MK-801 BUNDING TO NMDA RECEPTORS." J. S. Maruyama* and K. Batchley. Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

In well-washed membranes, glutamate increased each other's efficacies to stimulate [H]MK-801 binding to NMDA receptors, with no detectable effects in their potencies. Glutamate was virtually unable to enhance [H]MK-801 binding in the absence of glycine or in the presence of glycine antagonists, confirming the idea that occupancy of the glycine site is an absolute requirement for NMDA receptor activation. Conversely, the efficacy of glycine to stimulate [H]MK-801 binding was greatly reduced in the absence of glutamate and in the presence of glutamate antagonists. However, [H]MK-801 binding was stimulated to high concentrations (1 mM) of glutamate or glycine acting independently of each other. These low affinity components for glutamate and glycine are probably due to an agonist action of glutamate at the glycine site and of glycine at the glutamate site, respectively. In agreement with this idea, glutamate inhibited [H]glutamate binding, and glycine inhibited [H]glutamate binding, each at millimolar concentrations. Moreover, the potency and the low affinity component for glycine was reduced by glutamate but not by glycine antagonists, but not by glycine antagonists. These results indicate that 1) simultaneous occupancy of the glutamate and glycine sites is necessary to activate NMDA receptors, and 2) glutamate and glycine at millimolar concentrations can act as agonists at the high affinity site for the other compound in the NMDA receptor.

485.2

Depending upon concentration, magnesium can either inhibit or potentiate the binding of the NMDA channel blockers [H]MK-801 or [H]TCP. To determine how magnesium produces these effects, we examined the action of magnesium binding at synaptic sites and at sites of rat hippocampal NMDA receptor: glycine, glutamate, and the [H]TCP binding sites (i.e., phencyclidine receptor). At previously reported by others, magnesium produced basic effects on [H]TCP binding: magnesium exerted potentiating effects at lower concentrations and inhibitory effects at higher concentrations. Both effects required association of the glutamate and glycine binding sites. The inhibition of magnesium on [H]TCP binding was paralleled by an inhibitory effect on the apparent affinity of the [H]TCP binding site. The potentiating effects of lower concentrations of magnesium were associated with a concentration-dependent increase of both the association and dissociation rate of [H]TCP without change in either the Kd or Bmax of [H]TCP binding. Magnesium also increased the affinity of [H]TCP at concentrations comparable to those that increased [H]TCP binding. Magnesium did not modify NMDA-displaceable [H]glutamate binding. These findings support the idea that magnesium has two distinct effects on the NMDA receptor: 1) the apparently competitive interaction between magnesium and [H]TCP binding which likely corresponds to the well characterized channel blocking action; 2) a potentiation of NMDA channel activation likely mediated by the increased affinity of glycine binding.

485.3

NMDA receptor mediated neurotransmission is regulated by polyamines. To examine the direct interaction of polyamines with the NMDA receptor we determined the effects of the polyamines spermine (SPM) and spermidine (SD) on the binding of the NMDA channel blocker, [H]TCP. Both SPM and SD produced biphasic effects on non-equilibrium binding of [H]TCP. Both the potentiating and inhibitory effects of polyamines required agonist binding at both the glutamate and glycine binding sites. The potentiating effect, detected at concentrations of polyamines less than 30 mM, was associated with increases of both the association and dissociation rates of [H]TCP binding. The inhibitory effect of polyamines, detected at concentrations of polyamines greater than 100 mM, was associated with a decrease of the apparent affinity of [H]TCP binding. This study suggests that SPM and SD both have two distinct actions on the NMDA receptor: 1) the apparently competitive interaction between polyamines and [H]TCP binding which likely corresponds to the voltage-dependent block of NMDA channels described by MacDonald et al (1992); 2) a potentiation of the inhibitory effect due to an increase in the duration of which may correspond to the increased channel open frequency described by MacDonald et al.

485.4
THE NMDA ANTAGONIST [H]EPINIPRODIL BINDS TO HETEROGENOUS POLYAMINE-SENSITIVE SITES IN RAT BRAIN. H. Schoenaerken, S. Pignone and B. Ehrlich. Department of Biology, Synthetica Research Inc., P.O. Box 1601, Suite 500, 2000 Bayshore Drive, Durham, NC 27710.

The noncompetitive NMDA antagonist and cerebral anti-inhematic properties of ifenprodil have been described to binding to NMDA-like receptors in the hippocampus and NMDA receptor blocker, [H]TCP. Both SPM and SD produced biphasic effects on non-equilibrium binding of [H]TCP. Both the potentiating and inhibitory effects of polyamines required agonist binding at both the glutamate and glycine binding sites. The potentiating effect, detected at concentrations of polyamines less than 30 mM, was associated with increases of both the association and dissociation rates of [H]TCP binding. The inhibitory effect of polyamines, detected at concentrations of polyamines greater than 100 mM, was associated with a decrease of the apparent affinity of [H]TCP binding. This study suggests that SPM and SD both have two distinct actions on the NMDA receptor: 1) the apparently competitive interaction between polyamines and [H]TCP binding which likely corresponds to the voltage-dependent block of NMDA channels described by MacDonald et al (1992); 2) a potentiation of the inhibitory effect due to an increase in the duration of which may correspond to the increased channel open frequency described by MacDonald et al.

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485.5
7-CX-DEPENDENT AND INDEPENDENT EFFECTS OF POLYMANNOSIDIC ACIDS ON NMDA RECEPTOR INACTIVATION. L.S. Zukin, D.C. Javitt, R. Sirinar, M. Frisplante, Dept. of Psychiatry and Neurosciences, Albert Einstein College of Medicine and Bronx Psychiatric Center, Bronx, NY 10461.
Activation of N-methyl-D-aspartate (NMDA) receptors is regulated at distinct sites by molecules and processes in order to determine the differential effects of polymannosides and glycine on NMDA receptor activation, kinetics of [3H]MK-801 binding were measured in the absence and presence of L-glutamate, the non-competitive antagonist spermine and the putative competitive antagonist 7-CX. Two components of association were found: a fast component (not observed under control conditions) reflecting binding to the activated conformer complex and a slow component reflecting binding to agonist-associated closed conformations. Addition of L-glutamate (0.3-100mM) alone led to a dose-dependent 3-fold increase in total binding that significantly increase binding manifesting fast kinetics. Spermine significantly potentiated the degree to which L-glutamate stimulated total steady-state binding and also led to a large increase in the percentage of total steady-state binding that was attributable to the fast component of association. 7-CX attenuated the degree to which spermine increased the percentage of binding attributable to the fast component without affecting the spermine-induced increase in total binding. These findings suggest that the glycyne site primarily regulates the interconversion between open and closed conformations of the NMDA receptor complex whereas the spermine site regulates both the total number of non-desensitized complexes and the interconversion between open closed conformations.
Support: Pfizer/002130, Ritter Foundation (SRZ); NARSAD (CGL, RS); NAMI (RS).

485.7
Previously, we showed a dual effect of spermine (SPM) on the N- methion-D-aspartate (NMDA)-evoked [3H]NMDA release in hippocampal slices (Fachetti et al., Soc. Neurosci. 17:108-13). This was possible by distinct evaluation of the release burst directly by NMDA, in fractions with the agonist (NMDA fractions), and indirectly, in later fractions in the absence of the agonist (tail fractions), collected up to 25 min. We now demonstrate that MK-801, a blocker of synaptic transmission, discloses the enhanced SPM effect on the NMDA fractions, while the tail fractions-related effect is insensitive to TTX. In all experiments sliced hippocampi from Wistar rats (200-225 g) were used. Fractions (5 min each) were collected and fractional release (FR) expressed as percent of the [3H]NMDA present in the tissue at the corresponding times. 500 μM NMDA evoked 7.08±0.86% FR. TTX (2 μM) inhibited NMDA fractions of approximately 90% (P<0.01). 1 mM SPM, added inactive, restored FR to 3.71±0.37% (P<0.01). Moreover, the increasing effect of SPM on the tail fractions was not modified by TTX. 400 μM D(−)-2-amino-7-phosphonoheptanoic acid significantly prevented SPM effect (1.62±0.26% FR, P<0.01), in the presence of TTX. Our data suggest that SPM may modulate the NMDA-induced [3H]NMDA hippocampal release by acting on presynaptic NMDA receptors.

485.8
Concentration-jump experiments on voltage-clamped rat cortical neurones in culture have previously indicated an allosteric interaction between the glycyne- and glutamate-recognition sites on the NMDA receptor complex (Kemp & Priestley, Mol. Pharmacol., 39, 1991, 666-670) Thus, glutamate dissociates more slowly from its recognition site on the NMDA-receptor complex when glycyne is bound to the glycyne recognition site than when the low efficacy partial agonist, (−)-HA 966, is bound. We have extended these observations of interactions between these two co-agonist recognition sites by evaluating the effect of a range of agonists with differing levels of efficacy and affinity binding at one of the sites on the kinetics of agonists acting at the other.
The dissociation rate for glycyne in the presence of (−)-HA 966 is significantly faster than that seen in the presence of glycyne. Similar, but progressively smaller effects were seen with D-cycloserine, APDC (Aminoacyclopentaneacetic acid) and L-alanine. A reciprocal effect on glycyne kinetics was found to occur with several NMDA receptor agonists. Thus, the dissociation rate for glycyne was lowest in the presence of glutamate and became progressively faster in the presence of NMDA, quinolinic acid and cis-2,3-PDA (piperidinedicarboxylic acid).
These results suggest that co-agonists acting at the NMDA and glycine recognition sites on the NMDA receptor complex influence each other's affinity by an allosteric interaction the extent of which appears to correlate with the level of efficacy of the agonist rather than its affinity.

485.10
It is well established that glycyne potentiates responses mediated by the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor. We have characterized with [3H]glycyne these strychnine-insensitive binding sites in cerebral cortical membranes isolated from adult and 7-day-old mice. The binding was saturable, consisting of only one component in both age groups studied. The maximal binding capacity Bmax was significantly higher in immature mice than in adults. The binding constant KB was greater in immature mice, indicating lower affinity of the binding sites for glycyne. The binding was most strongly inhibited by glycyne itself, followed by zinc and β-alanine in both immature and mature cerebral cortex. β-Alanine was found to cause a mixed type of inhibition in glycyne binding. The binding of β-[3H]glycyne to cerebral cortical membranes was also saturable, consisting of one component. The binding constant KB for β-alanine was of the same order of magnitude as KB for glycyne, whereas the binding capacity Bmax was smaller. The binding of β-alanine was inhibited by glycyne, NMDA and glutamate but not by strychnine. The results point to the possibility that β-alanine could act at the glycyne modulatory site in the NMDA receptor complex of adult cerebral cortex. (Supported by the Emil Aallon Foundation, Finland.)
Differential Effects of L-Glutamate on [3H]-MK-801 Binding in PCP-Treated Weaning Rats Compared to Saline-Treated Controls. R. Sirota, Dept. of Psychiatry and Neurology, SUNY Buffalo, Buffalo, NY.

The developing rat brain is more susceptible to N-methyl-D-aspartate (NMDA) receptor-mediated neurotoxicity compared to adults. We have shown that in vivo microdialysis analyses of [3H]-MK-801 binding indicate lower density of binding sites in weaning rats treated postnatally with phencyclidine (PCP) compared to saline-treated rats; [3H]-MK-801 binding was used as a probe for NMDA channel activation. Here we report characterization of L-glutamate-induced NMDA receptor activation on [3H]-MK-801 binding in postnatal PCP-treated rats. Rats were treated with PCP (6 mg/kg) for 11 days. Controls received saline injections. [3H]-MK-801 bindings were measured in forebrain synaptosomal membranes prepared from PCP- and saline-treated animals, both in the absence and presence of 100 µM of L-glutamate (0-100 µM) with or without added glycine (10 µM). In the absence of added glycine, incubation in the presence of L-glutamate lead to a dose-dependent increase in specific [3H]-MK-801 binding both in the PCP- and saline-treated rats but neither mean EC50 values nor maximum [3H]-MK-801 bindings were different between the two groups. When binding was carried out in the added presence of glycine, maximal binding in the PCP-treated rats were lower than in the saline-treated controls. The mean EC50 value for L-glutamate-stimulated [3H]-MK-801 binding was reduced in both experimental and control rats but there was no apparent difference in the mean EC50 values between the two groups. These findings suggest PCP-treated rats in early postnatal period in rat alters the agonist-induced regulation of the NMDA channel activation.

Support: NARSAD, NAMI

Glutamate Sensitive Binding of [3H]-MK-801 to Intact Cerebellar Granule Cells. T.E. Murray* and V.J. Caldwell, Oregon State Univ., College of Pharmacy, Corvallis, OR 97331.

Efforts to label NMDA receptors in membrane preparations derived from rat cerebellum have yielded conflicting results. Although unsuccessful attempts to detect specific binding of [3H]TCP and [3H]-MK-801 in rat cerebellar membranes have been reported, a demonstration of binding of [3H]-TCP and [3H]-MK-801 labeling of cerebellar membranes has recently appeared (Ebert et al., Europ. J. Pharmacol.-Mol. Pharmacol. Sect. 208, 49, 1991). These cerebellar studding sites are distinct inasmuch as the affinity for [3H]-MK-801 is substantially lower than that of NMDA receptors in forebrain structures. To further explore the characteristics of cerebellar NMDA receptors, we have investigated the binding of [3H]-MK-801 in intact cultured cerebellar granule cells. The binding of [3H]-MK-801 was inhibited in a concentration-dependent manner by glutamate (1-300 µM). The glutamate inhibition of [3H]-MK-801 binding to intact granule cells was reversed by Mg2+ in concentrations ranging from 1 to 10,000 µM. Similarly, the glutamate-induced inhibition of binding was antagonized by the competitive NMDA receptor antagonist AP-5. Noncompetitive NMDA receptor antagonists such as MK-801, phencyclidine, and dextrophan produced biphasic effects on [3H]-MK-801 binding with low concentrations stimulating and high concentrations inhibiting binding. Equilibrium saturation analysis of the [3H]-MK-801 binding resulted in a Kd value of 77 µM and a Bmax value of 1.1 pmol/mg. Competition experiments indicated that glutamate exerted similar negative modulation of cerebellar NMDA receptors labeled with [3H]dextrophan. These results suggest intact cerebellar granule cells in culture express functional NMDA receptors.

Support: PHS Grant DA07218

Glutamate Sensitive Binding of [3H]-MK-801 to Intact Cerebellar Granule Cells. T.E. Murray* and V.J. Caldwell, Oregon State Univ., College of Pharmacy, Corvallis, OR 97331.

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Glutamate Sensitive Binding of [3H]-MK-801 to Intact Cerebellar Granule Cells. T.E. Murray* and V.J. Caldwell, Oregon State Univ., College of Pharmacy, Corvallis, OR 97331.

Evidence that high and low affinity AMPA binding sites reflect membrane-dependent stages of a single receptor. R. Hall, M. Kessler* and L. Jones, Center for Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

AMPA-type glutamate receptors are the primary mediators of excitatory neurotransmission in mammalian forebrain and are also thought to be central to the form of synaptic plasticity known as long-term potentiation (Staubli et al., Psychobiol. 18:377, 1990). Binding of [3H]-AMPA (DL-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) to membranes of cortical synaptosomes is dependent on the presence of potassium thiocyanate resulting in curvilinear Scatchard plots which could be resolved by regression analysis into two large affinity components (Kd<sub>1</sub> = 200-1000 nM approx. 50% of the total binding and Kd<sub>2</sub> = 10 µM approx. 10% of the sites). Treatment with 0.4% Triton X-100 resulted in a solubilized fraction which contained 74% of the sites recovered after solubilization. These sites were uniformly of the high affinity type with a Kd of 29 ± 5 nM. The total number of these sites was three to five times higher than the number of high affinity sites which was present in the starting membranes. Due to the solubilization of the high affinity receptor it was not possible to convert low affinity into high affinity receptors; it follows from this that the two affinity states represent interconvertible forms of the same receptor rather than separate receptors. The only mechanism for the presence of two affinity states appears to be greatly reduced in number in lysed synaptic plasma membranes; they accounted for only 1% of total binding in BPA. The evidence suggests that the high affinity receptor binds to a synaptic environment which keeps receptors in a low affinity state and that it is the lack of this factor during solubilization which allows receptors to revert to a high affinity state. (Supported by NS 21980 and AFOSR 89-0930).
485.17
RARE VISUALIZATION OF NMDA RECEPTORS: CHARACTERIZATION OF (+)-[3H]MK-801 BINDING TO THIN SECTIONS OF RAT BRAIN. W. Jacobson and G.A. Cotrell Department of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.
We have developed and characterized a method for the rapid autoradiographic identification of a competitive NMDA receptor antagonist. The compound (+)-[3H]MK-801 was one of the first NMDA receptor antagonists to be characterized. In the present study, we have developed a method for the rapid identification of NMDA receptor binding sites in thin sections of rat brain. The method involves the use of a high-speed tissue processor and the incorporation of (+)-[3H]MK-801 into the tissue sections. The method is rapid, sensitive, and specific for the identification of NMDA receptor binding sites in thin sections of rat brain.

485.19
MATERNAL VITAMIN B-6 DEFICIENCY ALTERS POSTNATAL DEVELOPMENT AND ZINC REGULATION OF HIPPOCAMPSAL NMDA RECEPTOR-ION CHANNELS. T.R. Guillemin and R.C. McGeer, Department of Environmental Health Sciences, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.
Vitamin B-6 (pyridoxine) deficiency during development results in reduced function of NMDA receptor-ion channels in the cerebral cortex (CTX) of 14 day old rats (Guillemin TR, Neuroscience. Lett. 121:207-210, 1991). The present investigation examines the effects of vitamin B-6 deficiency during development on the number of NMDA receptor-ion channels and zinc (Zn) uptake by the CTX. Long Evans rats (125-150 g) were fed either a normal (7.0 mg/kg pyridoxine, HCL-PH7) or marginally deficient B6 diet (0.7 mg/kg pyridoxine) for 2 weeks prior to mating. pups and progeny (weaned at 21 days of age) were fed the same diet through 14 days of age. NMDA receptor-ion channel binding was assessed at postnatal day (PND) 7, 14, 21, 28, and 36 as described (Guillemin TR, Fbd). The effect of the NMDA receptor agonist (10 μM) and zinc (Zn) on NMDA receptor-ion channel binding was also measured as a function of age. Cortical membranes from PND 7 rats were significantly more sensitive (IC50 = 0.9 nM) to the inhibitory effects of Mg than any other age tested (IC50 = 2.53 μM). There were no significant effects of diet. In normal rats, Zn showed a decrease in the IC50 (112 μM) at PND 7 to PND 21, and 25, and 36 (IC50 = 7.3, 3.0, and 4.5 μM, respectively). However, B6 deficient membranes at PND 7 required greater amounts of Zn to produce the same inhibitory effect as NMDA receptor-ion channel binding relative to age matched controls. These results suggest that vitamin B-6 deficiency may influence the postnatal development and regulation of NMDA receptor-ion channels in the rat cerebral cortex (Grass M # HD2093).

485.20
NMDA-RECEPTOR mRNA EXPRESSION IN THE CORTEX AND HIPPOCAMPUS OF CYCLING AND LACTATING RATS. R. Abrav, B. Atteni, G. E. Hoffman, and N. S. Smith, Department of Physiology, University of Pittsburgh, Pittsburgh, PA 15261.
Using cFos mRNA as a marker of neuronal activation, we observed an inhibition of cortical and hippocampal activation in response to NMDA, but not kainate, in lactating rats. This lack of responsiveness to kainate in lactating rats could be due to a decrease in the number of NMDA receptors in these areas. To examine this possibility, we measured the expression of NMDA-R mRNA in cycling and lactating rats. In situ hybridization was performed using a 14-nt labeled riboprobe recognizing 1.4 kb of the NMDAR1 mRNA. Areas of silver grains were analyzed in the parietal cortex, the piriform cortex, the CA1 region of the hippocampus, and the dentate gyrus, using the Optimas Image Analysis System. NMDA-R mRNA was abundant in all areas of the brain examined. Differences in NMDA-R mRNA expression between cycling and lactating rats were observed only in the CA1 region of the hippocampus (18% decrease in the lactating rats). Differences in NMDA receptor expression were not detected by Northern Blot analysis of tissue isolated from the cortex or hippocampus. These data suggest that the changes in NMDA-R mRNA expression in the lactating rat are very small, and that alterations in NMDA receptors in the regions examined might not explain the functional deficits in cortical activation in response to NMDA. Other possible explanations are that a small but key subpopulation of neurons is altered in the cortex, or that changes in NMDA receptors occur within the brainstem or spinal cord rather than in the cortex. It is also possible that suckling may inhibit activation of brainstem pathways necessary for cortical activation. Supported by NIH Grant HD14643.

486.1
BACLOFEN INHIBITS THE INCREASE IN CYCLIC AMP INDUCED BY HIGH FREQUENCY ELECTRICAL STIMULATION IN THE DENTATE GYRUS OF RAT HIPPOCAMPAL SLICE. M.J. Bonnarme and J.M. Savarey, Department of Pharmacy, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.
Activation of second messenger systems is implicated in the induction and maintenance of long term potentiation (LTP) and of synaptic plasticity. A high-frequency train (HFT; 100 Hz, 2 sec) of electrical stimuli was applied to the medial perforant path in the dentate gyrus of rat hippocampal slices. After HFT the dentate gyrus was cut from the rest of the slice and CA3 and CA1 levels of cAMP were determined by a protein binding assay. Each treatment group consisted of 3 slices from each of 6 rats. One min after HHT, CA3 levels increased to 240% of levels in slices that did not receive an HFT. Immediate post-treatment exposure of slices to baclofen (10, 100, 1000 μM) for 1 min resulted in concentration-dependent inhibition of HFT-stimulated CA3 levels. Levels of cAMP were reduced by 50% by 10 μM baclofen compared to levels produced post-HFT without baclofen. A high concentration (1000 μM) of baclofen post-HFT suppressed cAMP to basal levels. Pre-treatment of slices with 100 μM baclofen for 20 min before HFT also inhibited the baclofen-induced increase in cAMP to a similar degree as post-HFT baclofen. However, pre-HFT treatment with 10 μM baclofen did not produce any significant suppression of HFT-stimulated cAMP. These results suggest that the baclofen-sensitive GABA-B receptor can regulate stimulated cAMP levels in the dentate gyrus.

486.2
IN VITRO AND IN VIVO EFFECTS OF BACLOFEN ON CAMP OVERFLOW IN RAT CEREBRAL CORTEX. A.K. Knight, A.P. Whitton, and N.G. Brew, Dept of Pharmacology, School of Pharmacy, 29-39 Brunswick Square, London, UK.
The dual effect of baclofen upon cAMP production in brain tissue in vitro is well documented. The purpose of the present study is to elucidate and compare its effects in vivo.
In vivo experiments were performed by incubating aliquots of rat cortex chopped cortical slices for 10 min with either 10 μM forskolin or 100 μM noradrenaline to stimulate adenyl cyclase (AC) activity. In vivo experiments were performed by implanting a concentration dialysis probe into the frontal cortex, 100 μM forskolin was delivered at a flow rate of 2 μl/h, the dialysate was collected over two pulses, the first (31) to standardize the preparation, the second (32) for experimental manipulation. Radioimmunoassay was used to quantify the cAMP accumulated in the superfuse dialysis as a measure of AC activity. In vivo baclofen (0.01 to 100 μM) had no effect on baseline cAMP production but was found to dose dependently inhibit forskolin stimulated AC activity (pEC50 = 6.07 ± 0.29) and to augment the maximum stimulation obtained with noradrenaline (pEC50 = 5.94 ± 0.17). In vivo, 10 μM baclofen also failed to alter base line cAMP. However, when baclofen (10 μM) was administered simultaneously with forskolin during 30 min infusion, cAMP overflow was not reduced when compared to control 2B, but instead appeared to increase the stimulation produced by forskolin (S2/S1 ratio = 1.59 ± 0.57, control = 0.57 ± 0.07). The reasons for this are currently under investigation.
486.3 EFFECT OF (+)-BACLOFEN ON cAMP FORMATION AND SUBSTANCE P RELEASE FROM RAT SPINAL CORD. N.G. Bowery and M. Malcangio. Dept. of Pharmacology, School of Pharmacy, Univ. of London, London WC1N 1AX.

It has been proposed that baclofen may induce antinociception in rodents through activation on GABA_A receptors localized on the spinal cord. In this study we have investigated the effect(s) of (+)-baclofen on stimulated cAMP formation in rat spinal cord slices (350x350 μm) and on substance P release evoked by electrical stimulation (15 V, 10Hz, 1ms) of dorsal roots attached to spinal cord slices. (+)- Baclofen, dose dependent (10-100 μM), inhibited forskolin (10 μM)-induced formation of cAMP (maximal inhibition 56±5% at 100 μM) but this was not prevented by CGP 33548 (concentrations up to 1.5 mM). By contrast with cerebral cortical slices (+)-baclofen (1-100 μM) failed to enhance noradrenaline (100 μM)-induced formation of cAMP. Evoked release of substance P-LI was completely inhibited by (+)-baclofen (IC50 ~ 4.5 μM). Whilst GABA_A mediating analgesia may stem from a reduction in primary afferent neurotransmitter release it seems unlikely that an alteration in cAMP production is implicated.

486.5 ALTERATIONS IN TBPS BINDING IN THE LICODAINE-KINDLED RAT MODEL OF EPILEPSY. Marc S. Abel and Daniel E. Cashey. Department of Cell Biology and Anatomy, UHS/The Chicago Medical School, North Chicago, IL.

This autoradiographic study examined regional GABA_A receptors in chemically kindled rats. [3H]-Beryl bicyclophosphorothionate (TBPS), which binds to the GABA_A receptor chloride channel, was used as a ligand to identify GABA_A receptor complexes. Male Sprague-Dawley rats were injected daily with lidocaine (65 mg/kg, i.p.). Control rats received an equal volume of saline vehicle. Seizure activity was evaluated using the Racine Scale of 5 behavioral stages. The animals displayed a gradual increase in the severity of behavioral indices and by day 20 greater than 50% were in Stage 4 or 5. Midway through the injection regime some animals exhibited a gradual regression toward less severe behavioral stages; these animals were considered 'compensated'. After 25 injections the animals were killed, the brains removed and 30 μm sections incubated with 2 nM [3H]TBPS. Brain paste standards were prepared and included in the autoradiographic process. After appropriate exposure, the developed films were analyzed using a computer based imaging system. Binding was decreased in the subiculum, posterior lateral thalamic nuclei, hippocampal regions CA1 and CA3, and cerebellar nuclei in sections from kindled animals as compared to controls. Sections from rats that 'compensated' during the kindling process had normal or slightly elevated [3H]TBPS binding in those regions. These data support the hypothesis that alterations in the GABA_A receptor occur during the kindling process.


Under two-lever drug vs. no-drug discrimination procedures, the LDP training condition previously shown to be unique among benzodiazepines (BD) training conditions studied to date in that rats did not acquire the BD to pentobarbital reliably, even when training dose and tolerance to its rate-decreasing action were manipulated (Ablow and Griffiths, Environ. Pharmacol., 1989, 28, 20). Subsequently, generalization profiles for LDP, DP, and PB training conditions have been directly compared in Long-Evans rats in tests with a range of BD agonists and partial agonists, barbiturates, and other anxiolytics or sedatives. Rats in all three training groups generalized to BD, but greater selectivity in generalization to barbiturates and other sedative/anxiolytic compounds occurred in rats trained to discriminate LDP than DP. Rats trained to discriminate LDP failed to generalize reliably to any barbiturate or other non-BD sedative/anxiolytic, but the profiles for rats trained to discriminate DZP and DP were comparable. Among novel BD-receptor ligands, abacavir occluded drug lever responding under all conditions; zolpidem did so in the PB and DP conditions but not as reliably in the LDP condition. (Supported by NIH Grant DA 01431)


A depolarizing bicuculline sensitive response can be elicited in slices of hippocampus under conditions that induce a slow wave. This response is not evoked by the antagonism of excitatory amino acid receptor and seems to occur throughout the tissue. This response also occurs spontaneously in the presence of ABA, and does not appear to occur simultaneously throughout the tissue. It has been shown that there is some delay in the occurrence of the response between the distal CA3 dendrites and the subiculum. The delay across 2mm of tissue is approximately 150 msec suggesting there may be a synaptic component to the response and/or that there may be a loci where the response originates. The ability of GABAergic cells to produce depolarizing responses may be important in stabilizing GABAergic networks as well as limiting seizure-like activity. Whether activation of the postsynaptic cell through this mechanism may change the efficacy of synaptic contacts is a possibility, since calcium can enter the cell through these channels. When this occurs intracellular calcium increases, even when not paired with synaptic activation which produces synaptic modifications. As this response is most prominent in neonates, this would be a good mechanism for laying down a framework of GABAergic connections.


Baclofen application has resulted in facilitation of depression, and no effect in various thalamic nuclei. These separate results have led to the speculation that baclofen effects on GABA_A receptors demonstrate regional and laminar differences. We utilized a 16 channel rake electrode with subsequent SDT analysis and intracellular recordings to examine the laminar effects of baclofen following white matter stimulation in 16-22 day old rat visual cortex. Monosynaptic EPSPs in both layer II/III and V were unaffected by low doses (0.5-2.0 μM) of baclofen and equally attenuated by high doses (10-40 μM). Polykynetic EPSPs were selectively attenuated in layer II/III at low doses while layer V EPSPs were facilitated. Low dose baclofen selectively attenuated EPSPs in both layers. We conclude that baclofen, acting at presynaptic terminals, demonstrates differential laminar effects in immature rat visual cortex.
469.9
DECREASED NEURAL INHIBITION IN IN VITRO HIPPOCAMPUS AFTER 1 WEEK FLURAZEPAM (FZP) TREATMENT: AN INTRACLASSICAL STUDY. K.J. Stieglitz and E.L. Tietz. Department of Pharmacology, Medical College of Ohio, Toledo, OH 43699

FZP treatment significantly reduces GABA-mediated hippocampal paired-pulse inhibition (Xie and Tietz, 1991). Orthodromic EPSP-IPSP sequence and action potential (AP) burst frequency and duration, elicited by a 1 sec depolarizing current, was examined in vitro superfused slices 48 hr after 1 week FZP treatment. Male rats (175-200 gm) were offered FZP in the drinking water (100 mg/kg X 3 X 4 dy); selected 1 day after withdrawal of FZP. Control slices were treated (6 cells/5 rats) and control (7 cells/4 rats) slices had stable resting membrane potentials (RMP) -44.5±0.8 mV. Resting potentials > 30 mV (78.3±0.6 vs 93.9±1.6) and antidromic APs > 70 mV (82±2.9 vs 81±1.5) were held at <60 mV throughout the experiment. EPSP-IPSPs elicited by just subthreshold Schaffer-collateral stimulation were recorded (3 M KCl filled glass pipettes, 100-170 MΩ) from CA1 pyramidal cells (24°C). There was a significant (p<0.01) reduction in the amplitude of the FIPSP (1.4±0.6 vs 6.3±0.7 mV) and sIPSP (1.5±0.4 vs 6.5±0.7 mV) and an increase in EPSP amplitude (16.5±1.3 vs 5.4±0.8 mV). The small increase in burst duration and frequency (265±46.6 mS, 34±0.2£4 Hz vs 245±24.6 mS, 29±4.2 Hz) was significant (p<0.10). These data provide additional evidence regarding the nature of the impairment in GABA-mediated hippocampal function in chronic benzodiazepine treated rats. Supported by NIDA grant R01-DA04075.

469.11
GABA RECEPTOR ACTIVATION INDUCES GABA AND GLUTAMATE RELEASE FROM PROPERIC AREA. 1.0. Nakhabina, 1.0. Sokolov, 1.0. Blakaya, 1.0. K. Okajima, 1.0. K. Nishiyama, 1.0. Dept. of Psychiatry, 1.0. Biochemistry, 1.0. Molecular Pharmacology, 1.0. and Neuroscience, 1.0. Albert Einstein College of Medicine, Bronx, New York 10461.

The effect of y-aminobutyric acid (GABA) receptor agonists on release in vitro of radiolabeled GABA and glutamate was studied using a crude preparation of isolated nerve terminals (neurosecretory). GABA agonists were incubated (2 min., 37°C) with neurons prepared from hypothalamic (HYP), preoptic area (POA) and cortex (COR) tissues. GABA and the GABA receptor agonist muscimol, but not the GABA receptor agonist (-)-baclofen, stimulated [3H]-GABA and [3H]-glutamate release from POA but not HYP or COR neurons of male rats. These effects were partially inhibited by the GABA receptor antagonist picrotoxin and bicuculline. Muscimol-induced release of H-glutamate and [3H]-GABA was dependent on extracellular Ca2+. Muscimol failed to release N-H-GABA and N-glutamate from POA neurons of ovariectomized female rats. However, administration of estradiol and progestrone to ovariectomized female rats caused the appearance of muscimol-induced release of amino acids comparable to that obtained in male rats. Therefore, GABA receptor-induced release of amino acids is brain region-specific and modified by hormonal status.

469.12
GABA MODULATES CALCIUM AND MEMBRANE POTENTIAL OSCILLATIONS IN IMMORTALIZED HYPOTHALAMIC NEURONS. A.C. Dong, 1.0. Dept. of Neurology and Anesthesiology, UCLA School of Medicine, Los Angeles CA 90024

Cloned GHRH-secreting hypothalamic neurons (GT-1-7) in culture showed spontaneous oscillations in [Ca2+]i, as measured with fluorescence videomicroscopy. [Ca2+]i oscillations had a periodicity of approximately 160-260 seconds and the peak [Ca2+]i of each oscillation ranged from 100-500 nm. Ca2+ oscillations of individual cells were generally asynchronous, although some groups of cells showed synchronized oscillations. Spontaneous Ca2+ oscillations were reversibly abolished by 1µm TTX. Bath application of 1µM GABA evoked an increase in [Ca2+]i, in most cells (100-200 mV), and increased the frequency of Ca2+ oscillations; 10µM GABA evoked a greater increase in [Ca2+]i (100-500 mV) in some cells. Further increasing the frequency of Ca2+ oscillations was possible by increasing the frequency of Ca2+ oscillations. These responses to GABA were inhibited by 10µM bicuculline. Using the patch-clamp technique under current-clamp conditions, GT-1-7 cells showed oscillations of membrane potential between -60 and +20 mV, with a periodicity of 0.3-0.6 sec. These responses to GABA were inhibited by 10µM bicuculline. Under voltage-clamp conditions, GABA activated chloride currents which were blocked by bicuculline (1µM) and Zn2+ (100 µM). These results suggest that GABA mediated GABA receptors on GT-1-7 cells and that these responses inhibit GT-1-7 cell activity. Supported in part by a grant from the National Institutes of Health and by a grant from the Northern California Research Institute.

469.13
FEEDFORWARD INHIBITION IS REDUCED IN HIPPOCAMPUS AFTER CHRONIC BENZODIAZEPINE TREATMENT. X. Zeng and L. Tietz* Dept. of Pharmacology, Med. Coll. Ohio, Toledo, OH 43699

Recent studies alluded to the role of feedforward inhibition in mediating the effect of GABA agonists in CA1 region of hippocampus (Tietz, 1991). In this study, we investigated the role of feedforward inhibition in slices from 1 week chronic flurazepam (FZP) treated rats was examined in slices according to Lujic and Dunwiddie (1992). Slices (400 µm) were cut, 48 hr after treatment, from male rats (175-200 gm) offered FZP (100 mg/kg X 3 X 4 dy) in the drinking water. Control rats received saccharin water. Control and test population evoked by stratum radiatum stimulation (S2) on the subicular side of an extracellular recording electrode (2 µM NaCl filled glass pipette, 2-5 Mohm) were compared. The test pulse followed an orthodromic condition pulse (S1), subthreshold for a population spike, with interpulse intervals (IPI) of 10-100 ms. A knife cut was made through the alveus between SI and the recording electrode to block recurrent inhibition. Feedforward inhibition in control slices ranged from 100-555. Treated slices showed a significant (20-40%, p<0.01) reduction in feedforward inhibition and all levels of GABA-mediated inhibition are impaired in CA1 region of hippocampus of chronic benzodiazepine treated rats. Supported by NIDA grant R01-DA04075.

469.14

Neuronal cell lines provide a source of pure populations of neurons and allow the properties of many neurotransmitter receptors to be studied. None of the neuronal cell lines have been reported to express functional GABA receptors. In this study, there have been no reports of cell lines expressing functional GABA receptors. Using biochemical and electrophysiological techniques, we have identified neuronal GABA cell lines expressing functional GABA receptors. Membranes from immortalized hypothalamic (GT-1-7) neurons bound [3H]-GABA (IC50 0.5±0.2 µM) with high affinity and selectivity for GABA. This binding was displaced by GABA and related compounds, including (R,R)-baclofen, (S,S)-baclofen, muscimol, GABA-A agonist. The binding of [3H]-GABA was selective for GABA-A receptors. In addition, [3H]-GABA binding was blocked by bicuculline (1µM) and Zn2+ (100 µM). These results suggest that GABA receptor agonists on GT-1-7 cells lack y.subscripts. The neuronal cell line GT-1-7 contains GABA receptors. In this study, the presence of mRNA encoding GABA receptors was confirmed. PCR analysis of the cells revealed the presence of mRNAs encoding α1, β3 and β5 subunits but not α2, α4, α6, β2 or β3 subunits. These cells may provide a useful model system for the regulation of GABA receptor subunit expression.

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THURSDAY AM

GABA RECEPTORS: FUNCTION IV

1150

FRIDAY AM

GABA RECEPTORS: FUNCTION V

1340
486.15 PERIPHERAL BENZODIAZEPINE RECEPTOR BINDING IN HYPOTHYROID RAT VENTRICLES I. KRAGI* & R. SMIEHOROWSKI Dept. Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260

Previously we showed that the binding of tritiated benzodiazepine (3H) diazepam to brain membranes from hypothyroid rats was significantly increased compared to that of euthyroid controls (Krakowiak et al., 1987). The present study was undertaken to determine if a similar increase in 3H diazepam binding is observed in the peripheral benzodiazepine receptor of hypothyroid rats.

486.16 ANXIOLYTIC COMPOUNDS HAVE DIFFERENTIAL EFFECTS ON RAT EEG. K.L. Skirik and C.M. Brown*. Neurogen Corp., Brantford, ON T9L 0A7. Although currently available antianxiety agents are sedative, it is unlikely that sedation is a necessary property of this class of compound. Here, different anxiolytics were examined with a view to quantifying their effects on central arousal: as an example of a sedating compound, benzodiazepine; and a barbiturate, Sodium Pentobarbital, was compared with a recently developed Type I non-sedative, Zolpimid, a non-selective benzodiazepine, and a serotonin (5-HT1a) ligand, ipsipronine, which has been shown to exhibit sedative profile, was also examined. Anxiolytics were selected and the compounds were administered via a cannulated tail vein. Vigilance was maintained by using a randomized disturbing noise source. The EEGs were analyzed, demonstrated that all compounds except ipsipronine significantly increased power in the frequency band above 1 Hz. Ipsipronine, in contrast, decreased power at these frequencies. The lowest frequency range (1-4 Hz) was also affected differentially: the barbiturate and Zolpimid increased, but ipsipronine decreased, power at these frequencies; Zolpimid had no effect. These data show that all compounds affected central arousal but there was no common action which might reflect an anxiolytic action. The EEG change produced by the two most sedating drugs indicated that there was increased recruitment of thalamocortical circuits. Such recruitment occurs with reduced vigilance. Zolpimid acted similarly but more sedating, under circumstances in which the compound reduces anxiety, increased vigilance. In conclusion these results indicate, firstly, that there is no preclinical EEG signature for anxiolytics because the EEG is a measure of the effect of these compounds on central arousal. And, secondly, that sedation is another requirement for an anxiolytic action.

487.1 PROGESTERONE-INDUCED ELEVATION IN BRAIN 3a-HYDROXY-5a-PREGNAN-20-ONE IS ASSOCIATED WITH ANXIOLYTIC BEHAVIOR AND AN INCREASE IN GABA_A RECEPTOR FUNCTION. I. Bishop*, R. Klann†, and C.K. Kellough‡. Dept. of Psychology, College of the Holy Cross, Worcester, MA. The Department of Chemistry, Southwest Foundation for Biochemical Research, San Antonio, TX, and the Department of Psychology, University of Rochester, Rochester, NY.

We have previously reported that intracerebroventricular administration of the progesterone metabolite, 3a-hydroxy-5a-pregnan-20-one (allopregnanolone), elicited significant anxiolytic behavior in a dose-dependent and stereospecific manner (Brain Res, 513: 337-343, 1990). We have also found that the anxiolytic effect of diazepam was enhanced in progesterone female rats, relative to ovarianized (OVX) rats, as was the efficacy of a GABA-A-stimulated chloride (Cl-)-influx in cortical synaptosomes (Behav Neurosci, 105: 663, 1991). These results are consistent with the well-documented role of progesterone in the control of anxiolytic behavior, since the potent i.e. "positive modulators of the GABA_A receptor.

In the following experiments, the effect of a subcutaneous injection of progesterone (P), 0.6 mg/kg or 4 mg/kg in OvX rats on exploration of an elevated plus maze was examined. Blood serum and cortical concentrations of allopregnanolone were assessed. GABA-A-stimulated Cl- influx was determined in cortical synaptosomes from a subgroup of OvX females treated with vehicle or 4 mg of P. Significant anxiolytic activity was detected 4 hours after the administration of 1 or 4 mg of P, behavioral measures of anxiolytic efficacy were significantly correlated with blood and cortical levels of allopregnanolone. Treatment also increased the sensitivity of cortical synaptosomes to GABA (GABA_A), increased the EC50 and increased the maximal efficacy with which GABA stimulated Cl- uptake. Together, these data support the hypothesis that the psychoactive effects of P are mediated by the biosynthesis of 3a-hydroxy-5a-pregnan-20-one, and that allopregnanolone subsequently augments GABA_A receptor-mediated function.

487.2 DEHYDROANDROSTROSTERONE (DHEA) IMPROVES MEMORY IN NORMAL MICE AND MICE WITH AGE-INDUCED MEMORY DEFICITS. C.L. Blackwell*, D. Davy, and R.J. Barkham. Labs View Medical Center, Sylmar, CA 91342.

DHEA is a steroid formed in the brain where it is active at the GABA_A receptor complex. DHEA has been shown to enhance memory in avoidance paradigms. The purpose of these studies was to assess the effect of DHEA on working memory in the win-stay foraging paradigm. Briefly, a food deprived mouse is placed in a T-maze with a milk reward in both goal boxes. The mouse travels the maze and is allowed to choose the milk in one of the goal boxes. On the next trial, if the mouse remembers which way it went on the previous trial, it will go the opposite way. By increasing the delay between trials the length of time a mouse can remember can be reduced. DHEA improved performance and extended the time that mice could remember by up to 120 seconds up to 100 seconds. Young adult Swiss Webster mice are able to perform with delays up to 60 seconds. In 12 month old Swiss Webster mice, 70% had a memory deficit such that they could only remember at delays of 120 seconds. DHEA (0.005 mg/kg I.P.) increased the delay that they could remember to 180 seconds. Young adult Swiss Webster mice are able to perform with delays up to 60 seconds. In 12 month old Swiss Webster mice, 70% had a memory deficit such that they could only remember at delays of 120 seconds. DHEA (0.005 mg/kg I.P.) increased the delay that they could remember to 180 seconds. Young adult Swiss Webster mice are able to perform with delays up to 60 seconds.
487.3

PHYSIOLOGICAL VARIATIONS OF GONADAL STEROIDS MAY REGULATE GABA SYNAPTIC TRANSMISSION IN THE CEREBRAL CORTEX. M.J. Al-Dahan, M.H. Jalilian-Teherani and RH Thalman. Baylor College of Medicine, Houston, TX 77030

These results show that GABA, agonist binding in the cerebral cortex is significantly affected during normal variations in gonadal steroids that occur during the estrus cycle of the rat. Well-washed synaptic plasma membranes were prepared for filtration assays of the binding of the GABA, agonist (°)-baclofen. In addition, we examined the binding of the GABA, agonist (°)-8-OH-DPAT, a 5-HT, agonist that depends upon the same G-proteins as do GABA, receptors. (°)-Baclofen binding was lowest during estrus (72±10 fmol/mg protein) and then increased over metestrus (145±2) to reach a plateau by diestrus (215±30) and proestrus (237±31). In contrast, (°)-muscimol binding was at or near its maximum during estrus, and (°)-8-OH-DPAT binding declined slightly from proestrus to estrus, but then remained constant until the next proestrus. Saturation binding experiments showed that GABA, receptor density (Bmax) approximately tracked effects upon total specific binding in that Bmax was lowest during estrus. Intracellular recording methods will be used to assess the effects of this variation in GABA, binding upon receptor-channel coupling in slices harvested from animals in different stages of the estrus cycle. Supported by NIH grant NS-21713.

487.5

CHRONIC GABA TREATMENT DOWNREGULATES GABA, RECEPTOR COMPLEX AND α mRNA SUBUNITs IN MAMMALIAN CORTICAL NEURONS. M.K. Ticku, M.C. Mhatre and A.K. Mehta. Univ. of Texas, SC, Dept. of Pharmacology, San Antonio, TX 78284-7764

Chronic exposure of GABA was investigated on the binding of ligands to GABA receptor complex, GABA-induced [3H]flunitrazepam, [3H]Ro15-1788, [3H]Ro15-4513 (°)GABA and [3H]TSBPS by 35-45%. Chronic GABA treatment also decreased the GABA induced °-influx by 45% and GABA enhancement of [3H]flunitrazepam binding by 31%. All these effects were blocked by concomitant exposure of the neurons to GABA, receptor antagonist, 6-(1H)-benzothiazine and 5-(1H)-indole. The reduction in α,°1 mRNA may underlie alteration in GABA, receptor function, downregulation and/or uncoupling, observed following chronic GABA exposure of these neurons. Finally, GABA, induces downregulation, uncoupling and a decrease in αμ mRNA subunit are a GABA, receptor mediated event. Supported by NINDS grant NS35339.

487.7

Effect of Phosphorylation-dependent Rundown on GABA Receptor Pharmacology. M. Guenels, T.T. Gibbons, & D.H. Farb. Dept. of Pharmacology & Experimental Therapeutics, Boston Univ. School of Medicine, Boston, MA 02118

Repeated application of 30 μM GABA to cultured chick spinal cord neur- um in the whole-cell voltage-clamp configuration resulted in a progressive define, or "rundown" of currents induced by 30 μM GABA. Repeated apnon of 3 μM GABA did not decrease even after induction of rundown by repeated application of 30 μM GABA. Although 3 μM GABA did not normaltically elicit rundown, rundown of the 3 μM GABA response was observed when the response was potentiated by 50-ppm-30-μM-20-μM (5nS), or when a decrease was observed at the intracellular buffer. This result is consistent with a model in which GABA, activation induces rundown by promoting GABA, receptor (GABA,RI) depol Time. After rundown, there was a decrease in the maximum response to GABA, coupled with a leftward shift of the GABA EC50 from 167 μM to 6.4 μM. When APTP was present in the intracellular buffer, the decrease in the maximum GABA, induced current was prevented and the shift in the EC50 was decreased (253 μM). Rundown was also associated with a decrease in the maximum pretransmission enhancement of the GABA response by positive modulators such as 50 μM progesterone, pentobarbital, and chlorodazoxide suggesting a decrease in the allosteric coupling of GABA and steroid recognition sites of the GABA, receptor. In contrast, there was no change in the effects of negative modulators such as pregnenolone sulfate and zinc.

487.8


The GABA, receptor/chloride channel is an oligomeric polypeptide composed of homologous subunits (α,β,γ,δ,ε), each containing four transmembrane segments (M1-M4) and a ryosim loop between M3 and M4. The bovine GABA, receptor includes in this loop a consensus sequence for phosphorylation by protein kinase C (PKC) and is phosphorylated by PKC in vitro. Phosphorylation by PKC may regulate GABA, receptor desensitization. During "whole cell patch" intracellular recording, GABA, induced currents gradually decrease in amplitude. This "rundown" is slowed by ATP and enhanced by alkaline phosphatase in the pipette medium, and may result from GABA, receptor dephosphorylation (Chen et al., J. Physiol, 420:207). We now report that intracellular PKC slows rundown of GABA currents in cultured mouse cortical neurons and L02 cells transfected with cDNAs encoding the bovine α, β, γ, and δ subunits. In cortical neurons, peak responses decreased from 20 to 50% over 20-30 min. For transfected L02 cells, the normalized peak current in cells transfected with δ, in controls decreased to 52 ± 15% (n = 7) at 12 minutes, while cells with PKC showed little decrease (97 ± 23%, n = 5). These results suggest that the rate of GABA, current rundown is regulated by PKC, presumably by phosphorylation of the δ subunit.

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Rapid desensitization of the GABA<sub>A</sub> receptor has been observed in rat cortical (t<sub>1/2</sub>=2 and 533 ms) and mouse (155 and 235 ms) neurons. The Na<sup>+</sup> current decay is not due to Na<sup>+</sup> channel inactivation, and the receptor is rapidly desensitized. The rapid desensitization of the GABA<sub>A</sub> receptor is consistent with a high affinity (K<sub>3</sub>=4 nM) and a low affinity (K<sub>4</sub>=100 nM) ligand binding sites. The high affinity site is further characterized by a rapid (<t<sub>1/2</sub>=5 ms) and a slow (<t<sub>1/2</sub>=300 ms) component, which may be due to the presence of a high and low affinity receptor subtypes. The low affinity site is characterized by a single component with a <t<sub>1/2</sub>=300 ms. The rapid desensitization of the GABA<sub>A</sub> receptor is consistent with a high affinity (K<sub>3</sub>=4 nM) and a low affinity (K<sub>4</sub>=100 nM) ligand binding sites. The high affinity site is further characterized by a rapid (<t<sub>1/2</sub>=5 ms) and a slow (<t<sub>1/2</sub>=300 ms) component, which may be due to the presence of a high and low affinity receptor subtypes. The low affinity site is characterized by a single component with a <t<sub>1/2</sub>=300 ms.

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The GABA<sub>A</sub> receptor is subject to multiple subunits which have distinct functions for phosphorylation by various kinases, including PKA, PKC, and tyrosine kinases. The GABA<sub>A</sub> receptor is a member of the gamma-aminobutyric acid (GABA) receptor family, which is involved in the control of inhibitory neurotransmission. The GABA<sub>A</sub> receptor is modulated by several mechanisms, including changes in the expression of different subunit isoforms, which can alter the pharmacological properties of the receptor. The GABA<sub>A</sub> receptor is modulated by the activity of protein kinases and phosphatases, which can be regulated by various signaling pathways. The GABA<sub>A</sub> receptor is modulated by the activity of protein kinases and phosphatases, which can be regulated by various signaling pathways.
488.5 

Angiotensin (Ang II) is a peptide hormone that regulates many physiological effects on cholinergic and related indices in rat brain. PD (1-60 mg/kg, l.p.) decreased Ach levels by 50% in vivo PD may alter cholinergic function and NMDA receptor complex mediated responses.

488.6 

In many of its target cells, angiotensin II (Ang II) is known to regulate the density of its receptor in vivo. Recently, we have demonstrated that AngII produces a rapid decrease in AT1 receptors and a more delayed decline in the AT2 subtype in murine NIE-115 cells. In the present series of experiments we have examined the relative contribution of each AngII receptor subtype during agonist-induced downregulation. When AngII cells were exposed to AngII (10 nM for 20 min) there was a significant decrease in the density of both AT1 and AT2 receptors. Similarly, when cells were concurrently treated with AngII and CGP42112A (0.5 µM), a selective AT2 receptor antagonist, comparable downregulation of both receptor subtypes was observed. In contrast, when NIE-115 cells were treated with AngII in the presence of the AT1 receptor antagonist, DuP733 (2 µM), there was no decline in either receptor subtype. In fact, both AT1 and AT2 receptors significantly increased after this combination of agonist and selective antagonist exposure. These results demonstrate that AngII interaction with AT1 receptors is necessary for downregulation of both subtypes, and that selective stimulation of AT2 receptors appears to result in an upregulation of AngII receptors. As much, these data suggest that reciprocal interactions between AT1 and AT2 receptor subtypes may occur during agonist-induced downregulation in neuro-like cells. Supported by NS23986 and MH43787.

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Differentiated murine neuroblastoma NIE-115 cells possess both subtypes (AT1 and AT2) of membrane associated angiotensin II (AngII) receptors, which are similar in specificity, affinity, and molecular weight to those in rat brain. Functional slabodipilisation of NIE-115 cell membranes with the detergent CHAPS exclusively solubilizes AT1 receptors while AT2 receptors remain in the 105,000 x g pellet. Covalent cross-linking of [3H]-Angll to solubilized AngII receptors with the monofunctional cross-linker DSS specifically labeled two proteins, one minor protein of 102 kDa and one major protein of 69 kDa molecular weight. Affinity purification of solubilized AngII receptors with AngII affinity column resulted in elution of one high molecular weight band (110 kDa) and one low molecular weight band (66 kDa) when excess amounts of agonist were used to elute the proteins from the column. In contrast, when proteins were eluted with an excess amount of an agonist only one protein band eluted from the column with a molecular weight of 66 kDa. Covalent cross-linking of [3H]-AngII to affinity purified proteins derived from both sets of experiments resulted in specific labeling of one high (102 kDa) and one low molecular weight band (66 kDa) when chemical properties of the AngII receptor are similar in sequence of this protein corresponded to the sequence of PLC-a purified from guinea pig uterus. Rats were injected either with aldosterone (ALDO - 250 ug/day) or corticosterone (CORT - 50 ug/day). Control animals were injected with a propylene glycol vehicle. After 3 days, cerebellum, cerebral cortex, hippocampus, amygdala, anteromedian third ventricle (AV3V), and pituitary were dissected and proteins were analyzed by 2-D gel electrophoresis followed by immunoblotting with PLC-c antibodies. The results indicated that PLC-c was present in each of the brain regions examined. Moreover, in the brain the antigen detected two proteins of 60 and 70 kDa with different pl values, whereas in NIE-115 cells only the 60 kDa species was detected by the antisera. In AV3V, medial amygdala or pinnute, or not other brain regions, ALIDO or CORT altered the pl value of both the 60 and 70 kDa protein, and the relative amount of immunoactivity appeared to change in cultured cells as well. The physiological relevance of steroid regulation of neuronal PLC-c remains to be determined. Supported by NS23986, HD52857 and MH43787.

488.8 

Many of the cellular actions of angiotensin II are mediated by phosphoinositol (PI) hydrolysis and the attendant mobilization of intracellular Ca2+. Recently, we have reported that AngII receptors are coupled to a 60 kDa PLC-c in photo-stimulated PLC-c in cultured neuronal cells. In the present study, we have used two-dimensional (2-D) gel electrophoresis to examine the distribution of immunoreactive PLC-c in rat brain, as well as its regulation by corticosteroids. The PLC-c antibody was raised against a 60 kDa protein purified from rat liver. The N-terminal amino acid sequence of this protein corresponded to the sequence of PLC-c purified from guinea pig uterus. Rats were injected either with aldosterone (ALDO - 250 ug/day) or corticosterone (CORT - 50 ug/day). Control animals were injected with a propylene glycol vehicle. After 3 days, cerebellum, cerebral cortex, hippocampus, amygdala, anteromedian third ventricle (AV3V), and pituitary were dissected and proteins were analyzed by 2-D gel electrophoresis followed by immunoblotting with PLC-c antibodies. The results indicated that PLC-c was present in each of the brain regions examined. Moreover, in the brain the antigen detected two proteins of 60 and 70 kDa with different pl values, whereas in NIE-115 cells only the 60 kDa species was detected by the antisera. In AV3V, medial amygdala or pinnute, or not other brain regions, ALIDO or CORT altered the pl value of both the 60 and 70 kDa protein, and the relative amount of immunoactivity appeared to change in cultured cells as well. The physiological relevance of steroid regulation of neuronal PLC-c remains to be determined. Supported by NS23986, HD52857 and MH43787.

488.9 

Angiotensin (Ang II) is a peptide hormone that regulates many physiological functions including blood pressure, fluid and electrolyte homeostasis and neuronal activities of various target cells. Two types of ligands for Ang II have been described, AT1 and AT2. While both subtypes bind Ang II and its analog with comparable affinity, AT1 receptors have high affinity for Dup 753 and AT2 receptors have high affinity for PD 123195, PD 123319 (PD) and CGP 42112A. Ang II has been reported to interact with cholinergic and dopaminergic neurotransmitter systems. We investigated the AT1 ligand PD for its effects on cholinergic and related indices in rat brain. PD (100 mg/kg i.p.) given 30 min prior to sacrifice caused a dose dependent decrease of acetylcholine (Ach) content in rat striatum. The effect lasted for up to 6 h after a 30 mg/kg dose of the agent. PD in vitro did not alter the basal PD or choline uptake; thus it is unlikely to be causing the decrease in Ach levels by blocking choline uptake, suggesting that it may be releasing Ach. Basal cerebellar cGMP levels (a well characterized second messenger response modulated by the N-methyl D-aspartate (NMDA) receptor complex) were decreased by PD at 30, but not at 3 and 10 mg/kg. These results suggest that in vivo PD may alter cholinergic function and NMDA receptor complex mediated responses.

We characterized the binding of 125I-Endothelin (ET-1) to purified plasma membranes from whole rat brain and heart. Binding reactions were done in 0.5 ml of 137 mM NaCl, 10 mM HEPES (pH 7.4), 0.1% BSA, and the following protease inhibitors: soybean trypsin inhibitor (50 μg/ml), leupeptin (0.5 μg/ml) and pepstatin (0.7 μg/ml). To distinguish ET receptor subtypes we performed displacement curves using 0.2 nM 125I-ET-1 and increasing concentrations of either ET-1 or ET-3 (IC50 = 0.2 nM). Alamethicin (50 μg/tube) was shown to increase H-ouabain binding to rat brain (40%), but had no effect on 125I-ET-1 binding. Alamethicin was included in assays to characterize the effects of added MgCl₂ and guanine nucleotides. Scatchard analysis showed no effect of 10 μM MgCl₂ or 10 μM ET-1 binding Kd and Bmax (0.17 nM, 0.14 nM; 0.19 pmol/mg, 0.18 pmol/mg with or without MgCl₂, respectively). Increasing concentrations of either GTPγS or GDPβS had no effect on 125I-ET-1 binding to rat brain. Similarly, GDPβS (100 μM) had no effect on 125I-ET-1 binding to rat brain. In conclusion, both ET-A (heart) and ET-B (brain) receptors exist in a high affinity state in purified plasma membranes, which is not affected by the presence of MgCl₂ or guanine nucleotides such as GTPγS or GDPβS.

488.12 USE OF SELECTIVE ANTAGONISTS TO SUBSTANTIATE THE EXISTENCE OF A B₃-KININ RECEPTOR SUBTYPE IN SPINAL CARDIOVASCULAR CONTROL. P. Lopez, D. Rengo, M. Thakur and R. Couture. Dept. Physiology, Faculty of Medicine, Université de Montréal, Montréal, Canada H3C 3J7 and Dept. Pharmacology, Faculty of Medicine, Sherbrooke University, Sherbrooke, Canada J1H 5N4.

A role for kinins has been suggested in spinal cardiovascular regulation and autonomic control studies. We have demonstrated the presence of B₃-Kinin receptors in the rat spinal cord. In this study, selective antagonists were used to further characterize the receptor subtype involved in the intrathecal (i.t.) action of bradykinin (BK) on mean arterial pressure (MAP) and heart rate (HR) of the conscious rat. The i.t. injection of BK (81 pmol at T-9) elicited a transient increase of MAP and a longer lasting decrease of HR. The cardiovascular response to BK was significantly and dose-dependently inhibited by the prior i.t. injection (717-800 pmol, 3-5 min earlier) of three B₃ receptor antagonists (D-Arg[9Hyp,D-Phe₇]Leu²)-BK, D-Arg[Tyr,D-Phe₇]Leu²)-BK, D-Arg[Hyp,D-Phe₇]Leu²)-BK, but remained unaffected by pretreatment with similar doses of antagonists for the B₆ receptor (D-Arg[9Hyp,D-Phe₇]Leu²)-BK, D-Arg[9Hyp,Gly₇]Leu²)-BK) or the B₁ receptor (D-Arg[9Hyp,Glu₇]Leu²)-BK). Similarly, D-Arg[9Hyp,Glu₇]D-Phe₇]Leu²)-BK, D-Arg[9Hyp,Glu₇]D-Phe₇]Leu²)-BK, two non-selective antagonists for B₃ and B₁ receptors, failed to antagonize the cardiovascular responses to BK and displayed agonic activities at higher doses. Doses 10-fold higher (7.7 nmol) of Hoe 140, a potent antagonist for peripheral B₁ receptors, were required to inhibit the response to BK. These results suggest that BK may affect the cardiovascular system by acting on a B₃ receptor subtype in the rat spinal cord. [Supported by the MRC of Canada].

488.13 CHARACTERIZATION OF NON-OPIOID [3H]-DYN A-(1-13) BINDING SITES IN RAT HEART MEMBRANE PREPARATIONS. M. Dumont* and S. Lemarie. Department of Pharmacology, University of Ottawa, Ontario Canada K1H 8M5.

In the brain, Dynorphin A (DYN A) interacts with both opioid and non-opioid receptors, the stimulation of the non-opioid receptor resulting in a loss of tail flick reflex and hindlimb paralysis. In the heart, DYN A also includes non-opioid effects such as a passive inotropic effect and cardiac arrhythmia. Using membrane binding techniques, we have characterized the cardiac non-opioid DYN A receptor using [3H]-DYN A-(1-13). Binding assays were performed in two milligrams of membrane (0.8 mg protein) in 5 mM Tris-HCl (pH 7.4); 0.2% BSA at 4°C for 120 min followed by filtration through polyethyleneimine treated glass microfiber filters. [3H]-DYN A-(1-13) binding sites were sensitive to tryptic and scatchard analysis yielded linear plots with a Kd of 56.5 nM and a Bmax of 1.91 pmol/mg protein. In competition experiments, [3H]-DYN A-(1-13) binding was displaced by DYN A-(1-13), DYN A-(2-13) but not by DYN A-(1-8) and levorphanol. [3H]-DYN A-(1-13) binding was insensitive to e (DTG, (+)-3 PPP, (+)-SKF-10047) and PCP (TCP, MK-801) ligands. These results suggest that [3H]-DYN A-(1-13) labels a non-opioid DYN A receptor in the heart which upon stimulation may lead to cellular damage as seen in myocardial ischemia. Supported by HSFO.

488.14 PRESENSE OF FUNCTIONAL CORTICOTROPIN-RELEASE HORMONE RECEPTORS IN HUMAN Y-79 RETINOBlastoma CELLS. Maia C. Olimas* and Pierluigi Ovalli. Department of Neurosciences, University of Cagliari, Cagliari, Italy.

In human Y-79 retinoblastoma cells corticotropin-releasing hormone (CRH) produces a marked (60-fold) and rapid increase of adenyl cyclase activity. The concentrations of the peptide producing half-maximal (EC50) and maximal stimulations were 60 nM and 1.5-5 μM, respectively. The effect of CRH is GTP dependent, being minimal in the absence of added nucleotide and maximal at 10 μM GTP. The specific CRH receptor antagonist α-helical CRH 9-41 competively counteracts the CRH stimulation with a Ki value of 80 nM. Somatostatin and vasoactive intestinal peptide display sequence homology with CRH and high affinity for CRH receptors, mimick the effect of CRH with EC50 values of 10 and 11 μM, respectively. These results demonstrate the presence of functional CRH receptors in human Y-79 retinoblastoma cells and suggest that this cell line may be a suitable model in which to study the action of CRH on human retinal cell function.


CRH systems are involved in mediating various aspects of the stress response. CRH receptors in the pituitary mediate stress-induced ACTH release, whereas receptors in other brain regions likely mediate fear-related behavioral and physiological responses. The distribution and density of CRH receptors has been studied extensively in rats, but little work has been done in primates. We examined the regional distribution of CRH receptors in adult rhesus monkeys (Macaca mulata). Pituitary had the highest receptor density (302 fmol/mg protein). In brain, amygdala and cerebellum had significantly more CRH receptors than cortex, caudate, hippocampus, and hypothalamus. Scatchard analysis shows a linear binding affinity in pituitary, amygdala, and cortex (0.169-0.347 nM). The high density of receptors in pituitary and amygdala is interesting because these sites are major mediators of endocrine and behavioral changes associated with fear. Previously we showed that the pituitary-adrenal response in rhesus monkeys is not mature until animals are 12 weeks old. Quantitative autoradiographic studies were performed in pituitary and discrete regions from 0-10-week-old monkeys. In pituitary, no significant age-related differences were found. CRH receptors were evident in anterior and intermediate but not posterior lobes, and their density in the intermediate lobe was greater than in the anterior lobe. Binding sites in the anterior lobe had a “cluster-like’ distribution similar to that of corticotropes. CRH receptors in the intermediate lobe were more uniformly distributed, reflecting the distribution of corticotropin-releasing-producing cells in this region. Data from other brain regions will be presented.


Studies on several avian species have demonstrated that prolactin (PRL) and opioids (OP) regulate feeding, but neither the mechanisms nor the sites involved are identified. To address this question, we measured the density of specific [(125)I]-opiod ([125]I}-DOPPE (a deltamorphin ligand) binding sites in brain regions of adult male dark-eyed juncos (Junco hyperbola) by in vitro autoradiography. We found that specific PRL and OP binding sites are present in several hypothalamic regions (PRL: influndibulum (INF) > ventromedial hypothalamus (VMH) > paraventricular nucleus (PVN) > lateral hypothalamus (LH); OP: PVN > INF > VMH > LH). Some regions (VMH, LH) of the avian brain that contain a high density of PRL and OP binding sites control feeding behavior. Thus, these regions may constitute sites of action on this behavior of PRL or of a PRL-like molecule, and also of endogenous OP.

Several modulatory actions of the insect hormones FMRF-NH2 and related peptides on tension generated in the extensor tibiae muscle of the locust hindwing by stimulation of the slow excitatory motor neurons (SE) are now well established. Here we demonstrate modulatory effects of four newly sequenced neuropeptides isolated from the locust which are unrelated to FMRF-NH2. The following were examined: Tension, contraction rate, relaxation rate, E, and eip amplitude. The preparations were constantly perfused at 1.4ml/min and kept at 20°C. Peptides were applied as 100 μl aliquots into the superfusion line were they were diluted to a final concentration of 5μM. All peptides were isolated from extracts of brain complexes of Locusta migratoria. The following observations were made: 1) Locustatachychlin II (APF(9)FF-NH2) increased tension, the relaxation rate, eip amplitude and could induce a contraction at a stimulation frequency of 0.3 Hz. Effects lasted the presence of the peptide in the superfuse for several minutes. 2) Locustatachychlin III (APF(4)FYY-NH2) had identical effects. 3) Locustatrocnin III, a peptide belonging to a separate family of locust peptides which is supposed to stimulate locust oviducts in vitro, had short-lasting, but dramatic inhibiting effects on tension in this preparation, accompanied by a reduction in eip amplitude and a small depolarisation. 4) Locustaryminhibiting hormone (AWQDNLACW-NH2) which suppresses contractions of the hindgut and oviduct of locusts also had inhibiting effects on muscle, similar to Locustatrocnin III but at a higher dose (10μM). The results of these experiments suggest, that the hindgut of the locust may be subject to complex modulatory actions of different families of peptides. It is as such and as a experimental model particularly useful.

489.2 PEPTIDE IMMUNOREACTIVITY IN THE GENITAL GANGLION OF APLYSIA. S. B. Moffett* Dept. of Zoology, Washington State University, Pullman, WA 99164.

Cells and some other CNS neurons in Aplysia californica reproduction are well established, but neurons of the genital ganglion, located at the junction of the genital nerve with the sperm-oviduct, may also play a role. Indirect Immunofluorescence in wholemounts of the ganglia of young animals (30-50g) revealed intense immunoreactivity to Mytilus inhibitory peptide (MIP) in a quarter of the cells and SCP-1-like immunoreactivity in a non-overlapping population. MIP varicosities are densely distributed on the sperm-oviduct and ovotestis as well as in baskets of endings surrounding some non-immunoreactive cells. Antibodies generated against both catch relaxing peptide (CRP) and buccalin reveal several axons which enter the ganglion and project toward the ovotestis. The strategic position of the genital ganglion neurons and the inhibitory role that some of the peptides associated with the ganglion play in muscle contraction in other systems (Kobayashi and Munoka, 1990. Zool. Sci. 7:801; Kiss, 1991, Comp. Biochem. Physiol. 95C:207) suggest that genital ganglion neurons and bag cells may interact in coordinating egg-laying behavior.


Conantokin GV (CGV) is a neuropeptide isolated from the marine anemone Aiptasia vulgaris. CGV was originally isolated on the basis of its ability to induce a sleep-like state in young mice following ICV injection (Oliveira et al., Toxicon 23: 277, 1985). In older mice, CGV produced hyperactivity or "sleepy climber" activity, in which the hyperactivity alternated with periods of behavioral inactivity. We examined the effects of ICV injection of CGV in adult rats implanted with EEG and EMG electrodes. Animals were placed in an isolation chamber II with a red light and connected to a wheel to show free movement. Behavioral effects were visually assessed. CGV 0.11 nmol/rat had no effect on sleep or behavior. CGV 1.1 nmol/rat induced hyperactivity as well as ataxia. The EEG during this time appeared to be of greater amplitude than normal awake EEG, with overriding high amplitude waves, and could not be scored for sleep-wake stages. 5 of the 8 rats exhibited seizure-like EEG. The behavioral effects of the high dose appear similar to those seen in adult mice.


Vasoactive intestinal peptide (VIP) and related peptides are distributed in the CNS and peripheral nervous systems, and are found in high concentrations in the intestines. We previously showed (Arch. Int. Pharmacodyn. 305:14, 1990) that two VIP analogs induced a concentration-dependent contraction of guinea pig ileum, and that these analogs also induced a concentration-dependent secretion of endogenous acetylcholine (ACh). We now demonstrate that one peptide component of the venom of the Gila monster lizard Heloderma suspectum, which has a high homology to VIP, induced the release of [3H]ACh from a LM-MP preparation of guinea pig ileum. The helodermin toxin was approximately equal in potency to the most effective VIP, and the maximum evoked release of [3H]ACh was at 10 μM of each peptide. The evoked [3H]ACh released was ~ 0.3% of the total content of tritium in the tissue, which contrasts with 20 μM nicotine inducing ~ 0.4%, and 25 mM KCl inducing ~ 5%. These results show that helodermin toxin resembles VIP in specificity and ability to bind to receptors in LM-MP preparations which cause the secretion of endogenous ACh.
489.5  
ATRIAL NATRIURETIC FACTOR INJECTED INTO RAT SUBFORNICAL ORGAN BLUNTS VASOPRESSIN RELEASE INDUCED BY ANGIOTENSIN II. L. Steardo*, M. Iovine, J. Perez, N. Brunello and G. Raczynski, Center of Neuropharmacology, Institute of Pharmacological Sciences, University of Milan and Department of Neurology, 2nd Medical School, University of Naples, Italy.

The recently discovered atrial peptides are thought to be importantly involved in controlling body fluid homeostasis, both in animals and in humans. The proposed mechanism by which these peptides, collectively indicated as Atrial Natriuretic Factor (ANF), regulate salt–water balance have been the subjects of intensive research over the last few years. ANF has been recognized as a hormone of the brain, its receptors have been identified in close vicinity to those of angiotensin II in circumventricular organs, such as subfornical organs (SFO) and the organum vasculosum laminae terminalis. Via Purini 5, 8005 Freiburg, Germany.

We have recently shown that intraventricular infusion of ANF into rats has been investigated. ANF in doses dependent manner has been able to attenuate the enhancing effect of peripherally injected Ang II (192 μg/Kg/min) on plasma vasopressin levels. These findings support the hypothesis that ANF influences the Ang II effect on fluid balance and they indicate SFO as one of the main sites at which this interaction occurs.

489.7  

The central nucleus of the amygdala (CEA) is known to be involved in the regulation of the parasympathetic and passive coping response to conditioned and acute stresses. Neuroanatomical studies revealed that the majority of the corticotropin-releasing hormone (CRH) containing neurons in the CEA have direct connections with autonomic regulatory nuclei in the brainstem. A 7 min infusion of 30 ng CRH (in 1 μl CSF) into the CEA of freely moving male Wistar rats under stress-free conditions, led to an increase in heart rate, without changes in plasma noradrenaline (NA), adrenaline (A), and corticosterone. The CRH-induced tachycardia was effectively blocked by pretreatment with 1 μg α-\text{CRH}, a receptor antagonist of CRH. Both CRH infusion and pretreatment with α-CRH caused a slight behavioural activation. However, administration of α-CRH alone did not induce a behavioural activation. The shock-probe defensive burying test was used to examine the conditioned stress response. The results show that CRH infusion in the CEA failed to affect heart rate in a conditioned stress test. The behavioural activity showed a remarkable response towards a more active (sympathetic) response after CRH infusion, which was blocked by pretreatment with α-CRH.

The present results suggest that CRH induces a reduction of the parasympathetic output system and passive coping strategies. As CRH is given at the level of the cell body of the CRH neurons in the CEA, the results can be explained as an autoreceptor mediated inhibition of CRH release from the CEA with parasympathetic brainstem nuclei.

489.8  

Both bombesin, a putative peptide neurotransmitter, and its receptors have a wide and extensive distribution in the hypothalamic areas, including the arcuate (ARC) and the suprachiasmatic (SCN) nuclei. A total of 100 ARC and 174 SCN neurons were recorded and tested for bombesin in brain slices. Over 75% of ARC neurons were excited by application of 0.5 nmol of bombesin into the slice chamber. The effects of bombesin on SCN neurons, however, can be differentiated between neurons with different firing patterns, viz. it excited 75% of irregular firing neurons (n=113), while it only excited 17% and inhibited 34% of regular firing neurons (n=61). A dose-dependent effect of bombesin (from 0.005 to 5 nmol) on SCN neurons was also demonstrated. In 25 SCN neurons tested for both bombesin and gastrin-releasing peptide, 24 showed similar responses (67% were excited by both peptides). Pretreatment with Leu\text{1}-cyclic\text{[CH(NH)\text{2}]}-leucine\text{10}-bombesin, a bombesin receptor antagonist, blocked 67% of the bombesin effect in SCN neurons. The data implicate that bombesin may play a significant role in the regulation of selective SCN and ARC neurons.

489.9  
CRF STIMULATES CATECHOLAMINE RELEASE IN RAT MEDIAL PREFRONTAL CORTEX AND MEDIAL HYPOTHALAMUS, ASSESSED BY MICRODIALYSIS. Jan Lavicky, R. Don Brown*, and Adrian J. Dunn, Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-8936.

In vivo microdialysis was used to measure changes of extracellular concentrations of catecholamines in freely moving rats in response to administration of corticotropin-releasing factor (CRF). With dialysis probes in the medial hypothalamus, intracerebroventricular (icv) administration of CRF (17 or 330 pmol) dose-dependently increased dialysate concentrations of noradrenaline (NAd) and dopamine (DA) and all their measurable metabolites except normetanephrine (NM). Dialysate concentrations of serotonin could not be measured reliably, but those of its catabolites 5-hydroxyindoleacetic acid (5-HIAA) and indicanone (SK) were also elevated. DA and NE increased within the first two 20 min collection periods, and returned to baseline within 3 h. Similar data were obtained with dialysis probes in the medial prefrontal cortex after 17 or 167 pmol of CRF icv. IP administration of CRF (1 nmol) similarly elevated dialysate concentrations of NE, DA, 5-HIAA, and catecholamines catabolites except NM in both the medial hypothalamus and the medial prefrontal cortex. These results support earlier neurochemical data suggesting that CRF administered centrally or peripherally stimulates the release of both DA and NE in the brain.

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489.10  
EFFECT OF GALANIN ON TUBEROINFUNDIBULAR DOPAMINERGIC NEURONAL ACTIVITY AND PROLACTIN SECRETION IN MALE AND FEMALE RATS: C. Gopalakrishnan, K.A. McInerney, K.E. Moore, and R. Lookingland, Dept. of Pharmacol./Tox., Michigan State Univ., E.Lansing, MI 48824.

Tuberoinfundibular dopaminergic (TIDA) neurons terminating in the median eminence tonically inhibit the secretion of prolactin from the anterior pituitary. Prolactin, in turn, stimulates the release of dopamine from TIDA neurons and thereby regulates its own secretion. Central administration of the neuropeptide galanin is reported to increase prolactin secretion, but it is not clear if this effect is mediated by changes in the activity of TIDA neurons. In the present study, the effects of galanin on the basal and prolactin-stimulated activity of TIDA neurons were examined by measuring the ratio of 3,4-dihydroxyphenylacetic acid (DOPAC) to dopamine in the median eminence of both male and female rats. Intracerebroventricular (icv) administration of galanin or dopamine at a rate of 5 nmol (by 15 min) increase in plasma prolactin concentrations, but failed to alter the ratio of DOPAC to dopamine in the median eminence of either male or female rats. These results indicate that galanin-induced activation of prolactin release is not mediated by changes in the activity of TIDA neurons. On the other hand, galanin decreased the ratio of DOPAC to dopamine in the median eminence of both male and female rats whose TIDA neuronal activity was stimulated following experimental procedures that increase circulating prolactin concentrations (i.e., administration of prolactin, oestradiol, or bilateral ovariectomy). Taken together, these results indicate that the inhibitory effects of galanin on the activity of TIDA neurons is dependent upon the level of activity of these neurons in both male and female rats (supported by ADAHMA grant MH 42803).

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489.11
VAPORPRESSIN RELEASE FROM AMYGDALA IN VITRO. J. Baber, S. Marlo Pich, G.F. Koob and F.E. Bloom*. Department of Neuropharmacology, Scripps Research Institute, 1006 N. Torey Pines Rd., La Jolla, CA 92037.

Arginine vaporpressin (AVP) containing neurons have been shown to occur in limbic structures, including the hippocampus and amygdala, as well as in the classic magnocellular hypothalamic nuclei. We have investigated the release mechanism of AVP in vitro from dissociated rat brain regions. Micropipet amygdala, hypothalamus and somatohypophysis were each incubated in balanced Earle's salt solution for a total of 180 minutes at 37°C. After three 20-minute intervals for conditioning the cultures, perfusion with NMDA (60 mM) caused a marked stimulation of AVP release (amygdala; basal level 58 +/- 18 pg/ml, stimulated 90 +/- 23 pg/ml; hypothalamus; basal level 392 +/- 36 pg/ml, stimulated 831 +/- 91 pg/ml). No release was found with slices from the somatohypophysis. This stimulation effect is calcium dependent and was blocked in the presence of EGTA (10 mM). These results indicate that depolarizing agents induce the release of AVP-like immunoreactivity not only from the hypothalamus but also from limbic structures, such as the amygdala. This system may be suitable for evaluating the effects of other regulatory signals, such as the cytokines, on neuronal neuronepair release.

489.13
CHRONIC PEPTIDE T ADMINISTRATION PREVENTS NECROTICAL ATROPY RESULTING FROM NUCLEUS BASILIS LESIONS IN AGED RATS. D.J. Scoed1, J.M. Hug2, C.B. Phippot1, M.R. Ruff2 and G.W. Arandelus3. 1Dept. of Biology, Univ. of S Florida, Tampa, FL 33620, NICH, NIH, Bethesda, MD 20892, 2Center for Molecular & Behavioral Neuropsych, 1 Rutgers Univ., Newar, NJ 07102.

Vasoactive intestinal peptide (VIP) is co-localized in cholinergic terminals within neocortex. Since VIP has neurotransactive actions in vitro, it may be involved with, and/or be a precipitant of blood pressure changes in neocortex resulting long-term after cortical cholinergic denervation induced by nucleus basalis (NB) lesioning (Science 238:592, 1987). The two-fold purpose of the present study was: 1) to determine the long-term degenerative effects of NB lesions in the neocortex of aged rats, and 2) to test the ability of "peptide T" (PT), which has a potent peptide hormone, with VIP (7-11), to prevent any observed degenerative changes. Aged (20-21 month) male Sprague-Dawley rats received NB injections of isotonic acid (5 pg/ml in PBS) bilaterally. Immediately after lesioning, animals began receiving daily i.p. injections of either PT (1 mg) or vehicle solution. After sacrifice 5 months later, brain sections were stained with thionine for analysis of neocortical thickness (Layers II-IV) at the mid-prefrontal level. Compared to measurements from unoperated controls, NB lesioned animals given vehicle alone exhibited a significant 17.6% decrease in overall cortical thickness (p<0.002); there was a 13.2% decrease in Layer II (p<0.02), a 28.6% decrease in Layers III-V (p<0.001), and a 15.1% decrease in Layer VI (p<0.02). Chronic PT treatment completely eliminated the lesion-induced decrease in overall cortical thickness, as well as that within Layers II and VI. Also, the lesion-induced decrease in Layers III-V was significantly attenuated by PT (p<0.05). PT's action in preventing NB lesion-induced neocortical atrophy may involve a direct or indirect (glial-mediated) neuroprotective mechanism, presumably through VIP receptor activation.

OPIOID RECEPTORS: COUPLING AND BIOCHEMISTRY

490.1
SELECTIVITY PROFILES AND REGULATION BY GUANYL NUCLEOTIDES OF LIGAND BINDING TO μ, δ AND η OPIOID RECEPTORS IN BRAIN MEMBRANES. M. Williamson and J.M. Herry*. Biochemical Pharmacology Group, Paulus, Inc., Bothell, WA 98011

We have used specific radioligand binding assays for the μ, δ and η opioid receptors in whole brain membranes from guinea pig to establish selectivity profiles for various opioids and investigate effects of guanylyl nucleotide regulation of agonist binding. [3H]D-Ala2-D-Leu5-MePhe7-Gly8-val (DAMGO), [3H]D-Phe2-D-Trp6-D-Pen7-D-Leu8-Tyr9-Phe10-NH2 and [3H]ET-1 (100 nM) were used as specific radioligands for the μ, δ and η receptors, respectively. Assays were carried out in modified Krebs-Hense buffer containing protein inhibitors. Saturation isotherms revealed a single class of high affinity sites for [3H]DAMGO. Inhibition of [3H]DAMGO binding yielded the following Ki's: fentanyl (1.2 nM), nalorphine (0.76 nM), DPDPE (30 nM), naltrindole (3.2 nM), and noranolterenol (26 nM). Indirect Hill coefficients for converting ligands were near 1.0. High K(1) receptors were observed when the ratio of the Ki for s and a were 926 and 3.70, respectively. GTP-γ-S completely inhibited [3H]DAMGO binding with an IC50 of 0.35 mM. This receptor sensitivity for differentiation of μ receptors was observed when the ratio of the Ki for K(1) and a were 926 and 3.70. The Ki for DDPPE was found to be 2.2 nM for a single class of sites. USO488 was found to be highly selective for δ receptors since μ/κ=211 and δ/κ≈11,430. Norbinaltorphimine exhibited a K(1) of 0.35 mM and a K(2) of 0.39, indicating ligand heterogeneity in labeled sites. [3H]DDPPE was found to be highly selective for δ receptors, with μ/δ=168 and a/δ=500. Saturation concentrations of GTPγS or GDPβS reduced [3H]DDPPE binding. The inhibition of [3H]DDPPE at concentrations of 0.70 and 2.0 mM, and reduced the binding of [3H]DDPPE to 12% and 5% of control with IC50 of 0.15 and 0.92 μM. These results establish that guanylyl nucleotide regulation offers a new opportunity to convert μ to δ receptors to states of mixed affinity for agoists in guinea pig brain membranes.

490.2

PC12 rat pheochromocytoma cells are useful as a model system for neuronal development. In one subline of PC12 cells, PC12h, low levels of type-opioid receptors markedly increase in response to nerve growth factor (NGF). (Inoue, N. and Hatahaka, H. J. Biol. Chem. 252: 9238, 1977). However, we have been investigating the consequences of the appearance of opioid receptors in PC12h cells and examining concurrent changes in the expression of opioid-regulated genes. After 10 days of treatment with NGF, the number of opioid receptors (as measured by [3H]-diprenorphine binding), increases from a Bmax of 40 to 220 fmol/mg protein. We have previously demonstrated that the opioid receptors in this cell line can be down-regulated by etorphine, indicating that they are capable of responding to opioid agonists. In the current study, we examine the influence of whether opioid receptors in PC12h cells couple to inhibition of adenyl cyclase was addressed. Etorphine caused a dose-dependent inhibition of cAMP accumulation. This effect was reversed by naloxone. Furthermore, etorphine inhibition of cAMP accumulation was found to be non-NGF as well as NGF-treated PC12h cells. These data indicate that the opioid receptors on PC12h cells are coupled to adenyl cyclase, similar to δ receptors in other cell lines. (Supported by DA-06867)

Considerable evidence suggests that opioid receptors are members of the G-protein linked receptor family. For several G-protein coupled receptors, biochemical and immunological analyses have implicated transmembrane segments of the receptor as the sites of ligand binding. The relative potencies of the enantiomers of 2m-aminoethyl-6,14-e-n-d-ethenothetadrylporpine (I; close structural relative of etorphine) and a series of N-acetyl derivatives were assessed using a N-acetion/laxone/rat brain membrane binding assay. N-acetylation(I) had no effect on potency whereas the introduction of a hydrophobic N-phenylactyl(III), N-phenylpropionyl (IV), or N-phenylpropionate(VI) substantially increased binding potency 40-fold, and made these compounds as potent as etorphine (pK < 0.3 nm). The effect of these compounds on an electrochemically evolved potential from isolated neonatal rat spinal cord showed them to be potent agonists, with III as potent as etorphine, I, II, IV and V being less effective. The electrophysiological effects were reversed by naloxone, but upon naloxone wash-out, effects of the more hydrophobic compounds (III, IV, V) persistently returned. These results suggest that partitioning of these compounds into the hydrophobic core of the neuronal plasma membrane may play a vital role in their duration of action because the µ opioid binding site is located in the transmembrane region of opioid receptors. However, because III is a more potent agonist than IV or V, small structural changes must affect the ligand/receptor complex conformation that transmits binding information to the G-protein involved in the transmembrane signaling process.


Pretreatment of rat brain membranes with 15 mM HCl at pH 4.5 prior to assay at pH 7.4 produces the following modifications in signal transduction pathways that regulate adenyl cyclase (AC): 1) decreases stimulation of AC by Gp, 2) increases receptor-mediated inhibition of AC, and 3) increases inhibition by Na* and GTP of agonist binding to opioid receptors, with no effect on binding in the absence of Na* and GTP. In NG108-15 membranes, low pH pretreatment did not increase opioid stimulation of low KCl with or without GTP, or (U96,593) opiod of AC, in the presence of 120 mM NaCl. However, studies of the effect of Na* concentration on AC and GTPase activity in NG108-15 membranes have revealed that low pH pretreatment: 1) decreased basal AC activity in a manner that was inversely related to Na*, 2) increased opioid inhibition of AC at Na* concentrations below that required to support opioid inhibition of the enzyme in control membranes, and 3) decreased basal low KCl GTPase activity and increased opioid agonist stimulation of low KCl GTPase in a manner that was dependent on Na*. The common effect of low pH pretreatment on both AC and GTPase was to increase agonist effects at low Na* concentrations. These data suggest that a complex interaction between Gp, Gi, and inhibitory receptors is altered by low pH pretreatment.

Supported by PHS grants DA-02904 and DA-07246 from NIDA.

490.5 OPIATE RECEPTOR AGONISTS REGULATE PHOSPHORYLATION OF SYNAPSIN I IN SPINAL CORD-DORSAL ROOT GANGLION COCULTURES. L. Vogel, D. Saya, S.Y. Nah, LMH, NINDS, NIH, Bethesda, MD 20892; Dept. Neurobiology, Tel-Aviv University, Tel-Aviv, 69106 Israel

κ-Opiate receptor agonists were shown to inhibit adenylate cyclase activity as well as the voltage-dependent Ca2+ channels in spinal cord-dorsal root ganglion SC-DG cocultured neurons. (Neurochem J 25: 186, 1989; J Biochem. 264:347, 1989). We have, therefore, investigated the effect of κ receptor agonists on the phosphorylation of synapsin I, a synaptic vesicle-associated protein whose phosphorylation was shown to be regulated by cAMP, depolarization, and intracellular Ca2+ concentration. Depolarization of SC-DG cocultures (by high K+ or veratridine) as well as the addition of forskolin (which activates adenylate cyclase) leads to increased phosphorylation of synapsin I. The addition of κ-opiate agonists (such as U50488 and EKX) attenuated both the K+ depolarization- and forskolin-induced phosphorylation of synapsin I. The E50 obtained for U50488 was 5 and 1 μM, respectively. This attenuation by κ agonists was blocked by the opiate antagonist naloxone and 6-κ-opiate receptor agonist had a much weaker effect compared with κ. Similarly, κ-opiate agonists attenuated (by 30-50%) the high K+ or veratridine-induced phosphorylation of synapsin I in synapsosomes prepared from spinal cord. These results show that opiate ligands modulate synapsin I phosphorylation. Moreover, the data could explain the alterations in synaptic efficacy and reduction in neurotransmitter release observed following opiate treatment. (Supported by the Minerva and Schilling Foundations, the National Institute of Drug Abuse, and the German-tranche Foundation for Scientific Research and Development.)


Nuclear opioid binding sites have been discovered in NG108-15 cells. Marker enzyme analyses, electron and fluorescence light microscopy studies at attests to the purity of nuclear preparations. Immunohistochemical staining of cryostat sections of NG108-15 cells with an anti-opioid receptor antibody corroborated a nuclear localization. Nal-D-Leu-Phe, H-D-Ala2-NMe-Phe and H-D-Phe-Cys-Thr-Phe homologous binding (Kd and Bmax), H-D-Ser-D-Phe homologous competition curves, stereospecificity and kinetic data, satisfied criteria for the presence of δ opioid sites in purified nuclear preparations; neither μ- nor κ-specific binding were detectable. Agonists, Nal-D-Leu-Phe and Nal-D-Phe, bind with high affinity to nuclear membranes and with lower affinity to chromatin. In contrast, partial agonist H-D-Phe-Cys-Thr-Phe binding sites are in nuclear membranes, while low affinity binding was found in nuclear membranes. Gp(NH)2 sensitivity of H-D Pakle binding was detected in nuclear membranes but not chromatin. Opioid binding to nuclear membranes and chromatin was abolished upon cycloheximide treatment of cells. The results suggest that NG108-15 cells contain newly synthesized G protein-coupled δ receptors in nuclear membranes and uncoupled, internalized opioid sites in chromatin.


Previous studies from this laboratory have demonstrated the presence of κ opioid receptors on membranes from the mouse thymoma cell line, R1. Chronic exposure of these cells to the κ agonist USO, 488 produced changes in the receptor population, as indicated in ligand binding assays using the κ-selective agonist [3H]U69,593. The binding of 1 nM [3H]U69,593 to membranes from USO, 488-treated cells was reduced by as much as 55% in comparison to controls, which were treated with an equivalent concentration of USO, 488 for 15 min. The effect of USO, 488 was both concentration- and time-dependent, with the maximum decrease in binding observed after 24 hr of exposure. The decrease in [3H]U69,593 binding was due to changes in the Bmax value and a 2.3 fold increase in the Kd value. The effect of chronic exposure to membranes prepared from these cells, suggesting that κ and δ opioids did not produce a change in the number or affinity of κ opioid receptors. The R1.1 cell line will provide a model for characterizing the mechanisms of receptor downregulation in cells of the immune system. (Supported by USPHS DA04355 and DA07232.)

490.8 THE PRESENCE OF 3 OPIOID RECEPTORS ON COS-7 CELL MEMBRANES. T. Zalewska, E. Malatyńska, H.1. Yamamura. Department of Pharmacology, College of Medicine, The University of Arizona, Tucson AZ, 85704.

COS-7 cells are very often used in transient gene expression. They might be considered as part of an expression system for opioid receptor cloning. We report the presence on COS-7 cell membranes of high affinity binding sites for [3H]naltrindole (an antagonist of 5 opioid receptors). Equilibrium binding studies show that [3H]naltrindole labels a homogenous population of binding sites with a dissociation constant (Kd) of 72.6 pM and Hill value not different from unity. The measured receptor density (Bmax) is 17 fmol/mg protein. Naltrindole and U69,593 selective for δ opioid receptor, p-CI-DPDE, displaced [3H]naltrindole from its binding site with high affinity. The Kd value for naltrindole is 200 pM, and for p-CI-DPDE is 315 pM. The ligands p-DL- and p-Chloro-2-Indoleacetic acid were not only inhibited [3H]naltrindole binding at micromolar concentrations. This study demonstrates the presence of 3 opioid receptor sites on COS-7 cell membranes. It is concluded that these cells should be avoided for the expression of putative δ opioid receptor genes. Supported in part by NIDA grants.
490.9

KAPP-A-LIKE RECEPTORS ON ASTROCYTES STIMULATE L-TYPE Ca2+ CHANNELS. Peter S. Eriksson, Michael Nilsson, Maria Wågberg, Elisabeth Hansson and Lars Rönnbäck*1,2. Institute of Neurobiology 1 and Department of Neurology 2 University of Göteborg, Göteborg, Sweden. Cultured astrocytes from the cerebral cortex respond to κ-receptor stimulation with a substantial elevation of the cytoplasmic free calcium, visualized through the use of the fluorescent calcium indicator fura-2. The stimulation of κ-receptors using the agonist U-50,488H increased the level of calcium through a stimulatory effect on the transmembrane calcium influx. The transmembrane influx was dose dependent. Furthermore, it was completely blocked by the selective κ-receptor antagonist nor-binaltorphimine. The presence of L-type channels was verified by the use of Bay K8644. The effect of Bay K8644 was completely blocked by nifedipine, indicating the involvement of L-type channels. L-type channel coupled κ-receptors on astrocytes might represent a novel mechanism contributing to the depressant action of opioids on synaptic transmission via decreasing the availability of extracellular calcium necessary for presynaptic transmitter release.

490.11


To identify animal models differing in opioid receptor binding parameters and in heritable behaviors, we have examined the number of Bmax (nM) and affinity (Kd) of μ and δ receptor in whole brain preparations from 8 inbred strains of mice. TH-DAMGO (0.5-21 nM) and H-D-β-endorphin (0.04-4 nM) were used to characterize μ and δ receptors, respectively. Scatchard analyses revealed that the Bmax values for μ receptors ranged from 75 to 115 fmol/mg, with a rank order among the strains of BALB C, C3H/SHJ, C57/BL10, DBA, SJL, AKR. No differences in μ receptor affinity were observed among the strains (Kd = 3-4 nM). The number of δ receptors among the strains ranged from 55-83 fmol/mg, with a rank order (BALB C < C3H, C57/Bl6J, C57/Bl6D2, CBA < DBA, SJL < AKR) very similar to that observed for μ receptors. There were no differences among the strains in δ receptor affinity (Kd = 0.4-0.5 nM).

Statistical analyses revealed a significant genetic correlation between the number of μ and δ receptors in whole brain preparations from the 8 inbred strains (r = 0.88, p < 0.01). The strong genetic correlation between the number of μ and δ opioid receptors suggests that there may be common mechanisms involved in the regulation of the expression of these receptor subtypes.

490.14


Voltage-clamp recording was used to detect functional expression of opioid receptors in the Xenopus oocyte translation system. By injecting poly(A)+RNA isolated from 3 week-old rat striatum or whole brain, the oocytes often acquired intracellular Ca2+-related oscillatory responsiveness to DAMGO (μ agonist), DAOPH (μ agonist), DPDE (δ agonist) and U-50488H (δ agonist) at a concentration of 1 μM. These responses were very potent and observed after injection of mRNA, however, water-injected oocytes never responded to any of the agonists. After sucrose-density fractionation, RNA size class of about 8 kbase encoded these opioid receptors. In the oocytes injected with striatal mRNA, DAMGO and DPDE evoked the fluctuating current with higher probability and in larger amplitude than other agonists, whereas whole brain mRNA produced more DAMGO and U-50488H responses predominantly. The DPDE response of striatal mRNA-injected oocytes was antagonized by naloxone as well as the δ-specific agonists ICI 154192 and ICI 174864. DAMGO and U-50488H responses have not been characterized yet because of strong desensitizing property. These observations suggest that putative μ, δ and subtypes of opioid receptors mobilizing intracellular Ca2+ are expressed in Xenopus oocytes by rat brain mRNA.

490.19

CHARACTERIZATION OF A POTENTIVE OPIOID RECEPTOR IN THE PROTOZOAN STENTOR. T.G. Brabin*, S. Mora*. Department of Cellular and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

An understanding of the biochemical events underlying opioid-mediated processes has been hindered by the lack of selective agonists or antagonists active in mammalian systems. Therefore, a simple systems approach may prove beneficial in understanding the biochemistry of opioid-mediated events. The protozoan Stentor, an invertebrate with an opioid receptor and this simple organism could provide a useful system for the biochemical study of such receptors. We have previously shown that β-endorphin (β-ends) modulates a mechano-sensory conductance in Stentor with an EC50 of 3.0 μM. The result of this modulation is behaviorally apparent as a marked decrement in the probability that a cell will contract when mechanically stimulated. The effect of β-ends appears to be receptor mediated for several reasons. First, the β-end effect is mimicked by morphine and DAGO, but not by dynorphins or the enkephalins indicating a putative μ-like receptor. Second, the actions of β-ends, DAGO, and morphine are sensitive to equimolar concentrations of the opiate antagonist naloxone. Third, the effect of β-ends is pertussin toxin (PTX) sensitive. Stentor incubated for 24 hours in 200 μg/ml of PTX do not exhibit a depression in mechanical stimulus response probability when exposed to 1 μM β-ends or 20 μM DADO. This PTX treatment has no effect on control cells’ response probability and does not affect the cells’ electrical threshold. This suggests that these opioids act through a G-protein linked opioid receptor. Finally, Stentor exhibit tolerance with the continued exposure to β-ends, which work is aimed at identifying any endogenous opioid ligands as well as characterizing this receptor utilizing pharmacological and molecular techniques.
**490.15**

**IN VIVO IMAGING OF OPIOID RECEPTORS WITH [125I]JOXY, A NEW IODINATED NALTREXONE DERIVATIVE.**
M.J. Iadarola, L.S. Brady, M.V. Green, K.F. Bernard, B.R. de Costa and K.C. Rice. NAB, NIDR, 2CNE, NIMH, 2DNM, CC, 2CDBB, NIMH and 2LMC, NIDDK, NIH, Bethesda, MD.

We have synthesized and radiolabeled JOXY (de Costa et al., J. Med. Chem. in press) for use in single photon emission computed tomography (SPECT) imaging of endogenous opioid receptors in humans. JOXY, an iodo-derivative of naltrexone, functions as an antagonist and is an analog of cycloFoxy, a [125I]-labeled compound used in positron emission tomography (PET). Intravenous injection of 20 uCi to rats yielded highest levels of binding in striatum and thalamus, lesser binding in other areas, and the fewest counts in cerebellum. Counts were displaced to levels in cerebellum by pretreatment with (-) but not (+) naloxone. Autoradiography of brain sections after i.v. administration showed dense binding to striatal patches, laminae I-II of spinal cord, medial habenula and interpeduncular nucleus. We used an experimental gamma camera to obtain a brain scan of a distribution of [125I]JOXY in a living animal. A planar scan in the vertex projection revealed dense accumulation of radioactivity in the thalamus, midbrain and bilaterally in the caudate nucleus. This is one of a new method for imaging receptors in small animals in vivo and suggest that JOXY will be an effective agent for use as a ligand in SPECT studies of human opioid receptors.

**490.16**

**GLUCOSE METABOLISM AND OPIATE RECEPTOR BINDING IN THE DEAFFERENTED CORTEX IN MONKEY BY PET.**
D.J. Doubet, R.E. Carson, M.A. Changning, R. Saunders, NAB and R.E. C. Brain Imaging Center, NIMH; Nuclear Medicine Dept, NIH, Bethesda, MD.

To examine the role of the cortical opiate system, we studied glucose metabolism and P-18 cycloxy (CF) binding in the visual system of rhesus monkeys (N=4). The forebrain commissures and one optic tract were sectioned. CF distribution volume (DV) and glucose metabolic rates (CMRG) were determined in left and right hemispheres. The deafferented occipital and parietal and temporal areas had a significantly increased DV of CF in 18 and 20.6 and a significant decrease in CMRG (12, 8 and 6) compared to the contralateral side (p<0.05). Compared to normal rhesus, CF binding was significantly increased (25%) in the contralateral occipital, parietal and temporal areas and the ipsi and contralateral central and frontal areas, but glucose metabolism was normal. CF binding and CMRG were normal in subcortical areas.

This increase in DV of CF, likely due to an increase in unoccupied opiate receptors, in areas with decreased functional activity suggests that sensory deafferentation may play a role in the modulation of opiate function and that cortico-cortical connections are important in its regulation.

**CATECHOLAMINES: RECEPTORS III**

**491.1**

**ACTIVATION OF POSTSYNAPTIC BUT NOT PRESYNAPTIC DOPAMINE RECEPTORS BY DIHYDROXIDINE, A POTENT D1 AND D2 RECEPTOR LIGAND.**

Mottola et al. (Neuropsychobiology, 17:188) found that the D1 agonist dihydridoxine (DX) weakly bound to D2 receptors, using radio-labeled spiperone, a D2 antagonist, as a D2 receptor marker. Like D2 agonists, DX depressed tyrosine hydroxylase, but this was not altered by DX antagonists. In binding tests, we find that DX had higher affinity for D2 receptors (Ki=3.3 nM vs "H-U-86170, a [3H]DA agonist) than for D1 sites (Ki=38.5 nM vs "H-SCH 23938) and n2 sites (Ki=62.5 nM vs "H-histidine). In reserpinized mice, doses of 2.5 and 5.0 mg/kg p.o. stimulated locomotor activity similar to other D2 agonists. This effect was blocked by the D2 antagonist raclopride (1 mg/kg s.c.). However, unlike other D2 agonists, DX, 0.1, 1.0, and 5.0 mg/kg p.o. did not block amphetamine-induced locomotor activity in non-reserpinized mice. It did not alter firing rates of DA neurons in substantia nigra pars compacta (SNPC) with doses up to 5 mg/kg i.v. (apomorphine ED50=0.009 mg/kg i.v.), nor did it antagonize DA agonist effects in SNPC. In contrast, DX behaves like a D2 agonist with some affinity also for D1 receptors, similar to apomorphine. However, it does not stimulate or block the DA autoreceptor, a D2 binding site.

**491.2**

**EFFECTS OF DEPRENYL ON DOPAMINERGIC NEUROTRANSMISSION IN THE RAT MESOSTRIATAL SYSTEM.**

Deprenyl, a monoaminooxidase B inhibitor, has been reported to delay the initiation of levodopa therapy in patients with Parkinson’s disease (PD), although the slowing of the progression of PD caused by deprenyl is controversial. Deprenyl and its metabolite were undertaken in order to examine the influence of this drug on mesostriatal dopaminergic neurotransmission in control and 6-OHDA-lesioned rats.

We studied the chronic effects of treatment with deprenyl (0.25mg/kg, s.c. daily) for 5 and 30 days on striatal DA, DOPAC and TH contents, on D1 and D2 DA receptor densities and on [3H]spiperone binding in two groups of rats (Gi and G2) and compared them to controls by the delay of treatment. Each group was composed of shams (no lesion), sham + deprenyl (no lesion), 6-OHDA lesioned (no deprenyl) and 6-OHDA lesioned + Deprenyl (10). Striatal levels of DA and the metabolite DOPAC were determined by HPLC-EC; DA D1 and D2 receptors were measured by autoradiography, using [3H]SCH 23930 and [3H]Raclopride respectively as ligands; [3H]DA mRNAs were examined by in situ hybridization using a specific oligonucleotide; TH was studied by radioimmuno-histochemistry. The main obtained showed that:
- striatal DA levels were significantly increased (+70%) in sham-deprenyl animals of G1 group. In lesioned animals where more than 90% of DA neurons were destroyed, no consistent effect could be defined;
- the effects of deprenyl on D1 receptor expression are clear only after 15 days of treatment. These effects concerned, firstly, sham-deprenyl animals where striatal D1 receptor densities were significantly increased (+18% to 25%, p<0.001) compared to sham controls; secondly, lesioned-deprenyl animals where D2 mRNAs levels were increased about 12 to 17% (p<0.001) as compared to lesioned rats. No effect could be observed on striatal D1 receptors and TH levels.

**491.3**

**D1, DOPAMINE RECEPTOR TURNOVER AND mRNA LEVELS IN THE NEUROLEPTIC-RESPONSIVE (NR) AND NEUROLEPTIC NON-RESPONSIVE (NLR) Lines of MICE.**
Y. Qian, R. Hitzemann, G. Yount, J. White and R. Hitzemann. Dept. of Psychiatry and Neurobiology, Div. of Endocrinology, SUNY at Stony Brook, NY 11794.

The NR and NLR lines of mice differ >10 fold in their sensitivity (ED50) to catalepsy induced by neuroleptics with a high D1/D2 dopamine receptor activity profile. Recovery of pre- and postsynaptic D1 receptors was assessed by quantitative autoradiography following N-ethoxy carbonyl-2-ethoxy-1,2-dihydroquinoline (EDQ) blockade. Significant differences in receptor turnover were found between the two lines in the nucleus accumens (NA) and the caudate putamen (CPU), but not in the substantia nigra zona compacta (SNc) and the ventral tegmental area (VTA). For both lines, receptor production rates in the NA and CPU are higher than those in the SNc and VTA. For the NA and CPU, receptor production rates of the NLR line were lower than those of the NR line. Preliminary nuclear protection data show that such receptor turnover differences are associated with parallel differences in the D1 receptor mRNA level.

**491.4**

**USE OF BXD RECOMBINANT INBRED MICE TO DETECT GENOMIC MARKERS FOR NEUROLEPTIC SENSITIVITY.**

Our laboratory has used a selective breeding and standard inbred strains to investigate the biochemical and genetic factors associated with response and non-response to neuroleptic induced catalepsy (see e.g. Kanes et al., Soc. Neurosci. Abst. # 839.6, 1991). The study of recombinant inbred (RI) strains provides a unique mechanism for detecting linkage between the phenotype of interest and specific Quantitative Trait Loci (QTL). The strain distribution patterns (distribution of EPQ for the heterozygote of the BXD recombinant inbred series (RI)) has been determined. ED50's in the RI strains are normally distributed confirming that sensitivity to neuroleptic induced catalepsy is a polygenetic quantitative trait. The BXD was correlated with the SDP of 260 genomic markers mapped in the BXD's. This analysis indicates 14 markers significantly associated with the catalepsy SDP. Eight markers were found on chromosome 4, of which one were centered on FV-1, which is at on cm from the centromere. Correlated responses to neuroleptic catalepsy include the density of pre and post-synaptic D1 receptors, the density of midbrain DA neurons and the density of striatal cholinergic neurons. The effects of these responses and the associated QTL analysis will be presented.

**SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992**
491.5 CHOLINERGIC AND DOPAMINERGIC REGULATION OF NEUROPTIC RESPONSE IN SELECTED AND INBRED STRAINS OF MICE. H. Hitzemann, S. Kanes, Y. Glin and R. Hitzemann. Departments of Psychiatry, Pharmacology and Neurobiology SUNY at Stony Brook, 11734-8110 and YAMC, Northport, NY 11768. The successful selection of the neuroptic responsive (NR) and nonresponsive (NNR) mice (Hitzemann et al. 19911) and the marked variation in the neuroptic sensitivity among inbred strains of mice (Kanes et al. 1991) argues strongly that genetic mechanisms contribute significantly to the variance in neuroptic-induced catalepsy. The precise nature or extent of these mechanisms remains unclear. Given the well-established role of the striatal cholinergic system in regulating extrapyramidal dopaminergic responses, we have investigated striatal cholinergic cell number (choline acetyltransferase [ChAT] positive neurons) in the NR and NNR lines and in inbred strains. In comparison to the NNR line, cholinergic cell number is significantly increased 40 to 60% in the rostral but not caudal striatum. Similarly, we have found that among the inbred strains there is a significant correlation (r = 0.75 or better, p < 0.05) between the EDA for haloperidol-induced catalepsy and cholinergic cell number in both the rostral and caudal striatum. Focusing on the rostral striatum, there is no difference between the NR and NNR lines in D2 dopamine receptor density; however, for the inbred strains, D2 receptor density is inversely correlated (r = 0.72, p < 0.05) to cholinergic cell number. This difference between the selected and inbred lines argues that D2 receptor and cholinergic cell number can have independent genetic regulation within the striatum.

491.7 EFFECTS OF D2 AND D3 RECEPTOR ACTIVATION MEASURED BY MICROPHYSIOLOGY. M.P. Rosser, M.L. Kozlowski, R.L. Neve, and K.A. Neve. Screening and Biochemical Research, Bristol-Myers Squibb, Wallingford, CT, 06492.

491.8 INTERNAL ACCEPTOR SITE DIRECTS ALTERNATIVE SPLICING IN THE MOUSE D2 DOPAMINE RECEPTOR. Fishburn C.S., David C., Cameron S., and Fuchs S.S. Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.


491.10 CHARACTERIZATION AND REGULATION OF DOPAMINE D3 RECEPTORS. R.A. Cox, R.A. Henningsen, K. Duwe, R.L. Neve, and K.A. Neve. Molecular Center and Oregon Health Sciences University, Portland, OR 97213, and McLean Hospital, Belmont, MA.

The dopamine (DA) D3 receptor is a novel D2-like receptor whose pharmacological profile differs from that of DA D2 receptors. We have stably expressed D3 receptors in C6 glioma cells (C6-D3) and binding characteristics of the D3 receptor in [3H]spiperone [125I]iodobenzamide (which has high affinity (60-100 pM) for D3 receptors. Drug potencies in rat basal forebrain minus neostriatum (BF) are measured to these using tissue from rat neostriatum (NS), and recombinant D3 (C6-D3 and D2 (C6-D2) receptors. Two site analysis of the data indicated that a small population (11-20%) of binding sites in rat BF a pharmaceutical profile similar to that of C6-D3 receptors. For neostriatum, however, curves are best fit to only one.

The genes coding for six subtypes of dopamine (DA) receptors have recently been characterized and expressed in various cell lines. In this study we have compared the pharmacological properties of the human D3 receptor with the two alternatively spliced forms of the human D2 receptor, D2A (long) and D2B (short). The D2 receptor was expressed in hamster ovary cells and the D3 receptor in mouse fibroblasts. In vitro radioligand binding studies demonstrated that the benzamid analogs of [3H]spiperone and [3H]iodobenzamide bind with high affinity, saturability and low non-specific binding to the three DA receptor subtypes. The Kd (dissociation constant) values of [3H]spiperone for D2A, D2B and D3 were 1.4 and 1.43, and 1.43 and 1.43, respectively. As expected the binding of the benzamid analogs was regulated by sodium ions. In competition studies most of the agonists tested showed about equal affinities for the three DA receptor subtypes. However, remoxipride and spiperone had ten fold lower affinity, and some benzamid analogs showed two fold higher affinity for D3 than for D2 receptors. Among the DA agonists, the agonist derivatives displayed an up to fifty fold higher potencies at D3 receptors. Two affinity states for DA and quinpirole were observed in all three subtypes. It is concluded that in spite of the high level of homology in the transmembrane domain it is possible to obtain D2/D3 selective drugs that might prove useful for development of antipsychotics.
491.11

THE D3 ANTAGONIST, AJ76, INCREASES ACCUMBENS DOPAMINE AND SEROTONIN RELEASE: SIMULTANEOUS DETECTION ON LINE WITH BEHAVIOR. E. Eng*, E.T. Pelchan, R.T. Wochler, M.P. Feuer† and P.A. Broderick. Dept. Pharmacol., CNS Med. Sch., Colleges of Physicians & Surgeons, 138th St., NY, NY 10032, USA, CNS Res., The Upjohn Co., Kalamazoo, MI 49007, USA. AJ76 is a dopaminergic (DA-ergic), antagonist with a higher affinity for the D3 than the D2 (Nature, 1986:347:146; 1990). AJ76 is a weak stimulus due to DA antagonist activity (Svensson, et al., J. Neural Transm. 65:1; 1986), with preferential activity at the nerve terminal (Piercey and Lum, Eur. J. Pharmacol. 282: 215; 1990). AJ76 increases Ca2+ dependent DA release (Waters et al., J. Pharmacol. 187:425; 1990). We now study the effects of AJ76 on concurrent accumulation of DA and serotonin (5-HT) with in vivo electrochemistry. Simultaneously, activity patterns were studied by infrared photocell beam detection. Searate working microelectrodes (Broderick, P.A., Brain Res. 495:11; 1989) were implanted in NAcc, under Na pentobarbital anesthesia; 9-15 recovery days were allowed. The results show that AJ76 increased both DA and 5-HT release, while concomitantly increasing locomotor activity, rearing behavior, stereotypy and agoraphobic inhibitory behavior (central ambulations). While DA release remained above baseline in the second hour, 5-HT release in concert with behaviors, decreased to baseline values or below. Thus, AJ76 maintains an upregulated DA release while 5-HT and behavioral stimulant components subside, without sedation. Consistent with electrophysiological data (Huang, et al., CNS Research and Technology, 13:15; 1989), the data demonstrate that AJ76 may have an autoreceptor antagonist mechanism for 5-HT. [Supp: in part Upjohn Co. RF #7-6207].

491.13

COMPARISON OF DOPAMINE-D2 AND D3 AGONISTS AND ANTAGONISTS ([+)-JUH-223] AT SYNTHESIS MODULATING DA AUTORECEPTORS IN VITRO. M.P. Galloway*, M.J. Keegan and S. Bertoluci. Cellular & Clinical Neurobiology, Wayne State Univ Sch Med, Detroit, MI 48207 USA. The recent discovery that mRNA levels for the dopamine (D3) receptor subtype are sensitive to 6-OHDA lesions suggests that expressed D3 receptors may function as DA autoreceptors. Since DA autoreceptors are defined functionally according to the functional properties (i.e., DA synthesis, release, and cell firing), we have investigated the effects of D3-prefering agonists (7-OHDPAT) and antagonists ([+)-JUH223, (+)-JUH226) on the DA synthesis modulating autoreceptor. Using the accumulation of DOPA in the presence of the dopamine decarboxylase inhibitor NSD1015 as a measure of tyrosine hydroxylase in K+ stimulated (30mM) striatal slices, we found potent and efficacious agonist activity of monochlorophenylalanine such as 7-OHDPAT and 5-OHDPA. The DA agonist effects (0.3mM) were fully reversed by (+)-JUH226, (+)-JUH223, clozapine, haloperidol, and eticlopride. The calculated (Gaddum equation) equilibrium dissociation constant (Kd) for the antagonists at the DA autoreceptor were determined in the presence of 7-OHDPAT. HAL: 3mM, ETH: 15mM, (+)-JUH226: 112mM, clozapine > 427mM, and JUH226: 1.6mM. In the presence of either 5mM K+ or forskolin (10mM), Kd values for (+)-JUH232 or SULP increased approximately 10 fold suggesting that anagonist potency in vitro is enhanced by elevated levels of extracellular DA. Comparison of these data with kinetic parameters (Kd) derived from ligand binding studies may elucidate a functional role for D3-DA receptors. Supported by MI Dept of Mental Health and NIDA RO1-04120 (MPG).

491.14

IODINATED 7-OH-DPAT A POTENTIAL LIGAND FOR THE D3 DOPAMINE RECEPTORS. C. Foulen, M.P. Kung, J. Billings, V.A. Boundy, P.B. Molinoff and H.F. Kung*. Dept. of Radiology, Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104. Recent success in cloning the D3 dopamine receptors and defining its properties has generated significant interest in developing new ligands for in vivo and in vitro studies of the receptor subtype. Thus, the DA-ergic system is a useful as pharmacological tools and therapeutic agents. Quinpirole, AJ76, JUH225 and 7-OH-DA have been reported to be D3 selective. Among them, 7-OH-DA appears to show high affinity and selectivity for Ki of D2/D3=100). 7-OH-DA was synthesized according to the published method, and iodination produced the corresponding 6-iodo-7-OH-DA (racemic). Spectra, HPLC and elemental analysis are consistent with the expected chemical structure. In vitro binding assays using [3H]NCO 296 showed similar affinity for D2 (rat striatal membrane) and D3 (membranes are from STg cells infected with a recombinant baculovirus containing the rat D3 gene) The Ki values for D3 receptors were 0.7±0.18 and 12±1.8mg/ml for 7-OH-DA and 6-I-7-OH-DA, respectively. While the Ki values for D2 were 156±17 and 264±33mM, respectively. Adding the iodine atom clearly decreases the D3 affinity of 7-OH-DA by 17-fold. Nonetheless, the 6-I-7-OH-DA retained D3 selectivity. Radioiodination was accomplished by reacting sodium [3H]iodine in the presence of peracetic acid as the oxidant. In vivo biodistribution studies in rats indicated that the 6-I-thyroxine-c1727 sodium the brain barrier easily. Further studies are needed to validate the nature of brain localization. It appears that the 6-I-thyroxine-c1727 may be a useful agent and warrants further evaluation. Acknowledgement: Support from PHS (NS-24538 and NS-18591) and Region Centre (61256012), France.

491.15

CLONING OF THE HUMAN DOPAMINE D3 RECEPTOR AND ITS EXPRESSION AND PHARMACOLOGICAL CHARACTERIZATION IN A VARIETY OF MAMMALIAN CELL LINES. R.A. Horlick, D.S. Conklin, D.G. Gregoraitis, G.M. Cooke, K.D. Cornell, S. Hutton, E.D. DeFeo and B.S. Schrag. Dept., The DuPont Merck Pharmaceuticals Co., Wilmington, DE. 19880-0400 Dopamine receptors are thought to play a key role in the clinical manifestations and treatment of psychoses including schizophrenia. The discovery of multiple dopamine receptors offers new tools for the development of receptor subtype-specific ligands that will effectively ameliorate psychotic symptoms without unwanted extrapyramidal or neuroendocrine side-effects. The dopamine D3 receptor, identified by molecular cloning (Nature 347:146, 1990), has an unique localization in the brain, its presence in areas of the brain linked to the limbic systems and its absence from the pituitary make it a likely target for antipsychotic drug activity without the generation of undesirable side-effects. Here we describe the cloning of the human dopamine D3 receptor from human nuclear accumbens mRNA using PCR. The receptor was introduced into CHO cell lines: CHO (Chinese Hamster ovary epithelial cell), 293 (Human embryonic kidney cell), Ltk- (murine fibroblast cell), and GH4C1 (rat pituitary lactotroph cell). The relative levels of expression of the D3 receptor in CHO, 293 and Ltk- cells are as follows: 12,500, 625, and 250 fmol/nl of protein, respectively. The receptor exhibited [3H]sperone binding which was saturable, membrane concentration dependent, temperature dependent, stereospecific and of high affinity. These four dopamine D3 receptor lines exhibit dopaminergic pharmacology with the appropriate rank-order profile for dopamine receptors.

Middle cerebral artery occlusion (MCACO) results in an acute increase in sympathetic activity in Wistar rats, and a decrease in spontaneously hypertensive rats (SHR). There is evidence to suggest that these autonomic changes are due to damage of the insular cortex (IC). The IC was selectively lesioned, using D.L homocysteic acid (DLH; 1 mM), in urethane-anesthetized Wistar and SHR rats. Arterial pressure (AP), heart rate (HR), renal sympathetic nerve discharge (SNR), and ECG were measured in male SHR (12) and Wistar (12) rats, following a 500 ml injection of DLH or saline control into the IC. Initial AP and SNR were not significantly different in SHR (85 ± 3 mm Hg; 27 ± 8 µV/s) and Wistar rats (78 ± 3 mm Hg; 26 ± 9 µV/s). Wistar HR (157 ± 11) was initially higher than that of SHR (319 ± 10; p<0.05). In the SHR, AP rose continuously in control animals, and was significantly greater than initial, as well as all lesioned rats, by 3 hr after injection. SNR did not change in SHR control animals, but declined significantly in IC lesioned rats, by 3 hr after DLH injection. HR increased in both groups of SHR. The pattern of change was different in Wistar rats. IC lesion, in Wistar, resulted in a significant increase in AP by 4 hr after DLH injection. There was no change in the AP or SNR of Wistar controls. HR increased in both Wistar groups. IC lesion increased the pressor response in Wistar rats. This suggests that the IC is at least partially mediating the autonomic responses to MCACO. (Supported by HSFC and HSFO)

492.2 CARDIOVASCULAR RESPONSES TO NEURONAL ACTIVATION OF THE EXTENDED AMYGDALA IN ANESTHETIZED AND CONSCIOUS RATS. A.J. Gelasena*, N.E. Copeland, G. Drotlef and H. Buchwald. Hypertension Unit, University of Ottawa Heart Institute, Ottawa, and Unité d'Hypertension du CHUL, Université Laval, Québec, CANADA.

The bed nucleus of the stria terminals (BNST) and sublenticular substantia innominata (SLI) are considered to be important relay stations of the hypothalamus and their involvement in cardiovascular control has not been studied. We explored these areas systematically in 27 spontaneously breathing, urethane-anesthetized Wistar rats (12 rats from where changes in arterial pressure (AP) and heart rate (HR) could be observed by injection of 20 ml glutamate (Glu, 0.5M). Injections into 84 of the 130 histologically verified sites in the BNST and SLSI were followed by 80 ± 0.7 s latency by depressor responses ranging from -4 to -33 (mean ± 13.3 ± 0.8 mmHg, accompanied by variable changes in HR. Pressor responses (4.5 ± 1.3 mmHg) were found after stimulation of only three sites; 43 sites were not responsive. Depressor responses evoked by stimulation in 17 sites in 6 rats before and during paralysis and artificial ventilation were not significantly different (p>0.05).

A different group of 10 rats was instrumented for bilateral Glu injections (200 nl, 0.1M) into the lateral BNST and for the recording of AP, HR and regional blood flow (using pulsed Doppler flow probes) in the carotid artery. Decreases in AP (10±2±1.6 mmHg) were elicited exclusively, accompanied by small (9.8 ± 4.4 bpm) increases in HR and renal conductance (11.1 ± 2.2%) and larger (32±7±1.8%) increases in hindquarter conductance, whereas mesenteric conductance decreased by 7.9±7.9%.

These results suggest that the BNST and SLI may participate in the cardiovascular correlation of the hypothalamus. (Supported by the Heart and Stroke Foundations of Ontario and Quebec, and FRQ/S)


Oclusion of the middle cerebral artery (MCACO) at the level of the meningeal sinus in anesthetized rats mimics the cardiovascular abnormalities that are observed in patients following focal cerebral ischemia. The stroke-induced autonomic symptoms may be due to pathophysiological changes that occur centrally following brain ischemia. MCACO or sham-MCACO was done in neurosurgical anesthetized male, Wistar rats. Five days after MCACO, the animals were paired and anesthetized before immunohistochemical demonstration of neuropeptide-Y (NPY), neuropeptide (NPY-ENK) and neurotensin (NT) using the diaminobenzidine reaction. A computerized-microscopic image system was used to quantify differences in staining between the two sides of the MCACO brains and between the MCACO rats and sham animals. A three-fold increase in the density of NPY labeled fibers and terminals was observed in the insular cortex and basolateral amygdala ipsilateral to the MCACO compared to that of the contralateral side and sham-operated controls. In the central nucleus of the amygdala, two to three-fold increases in NT and NPY-ENK labeled fibers and terminals were also observed ipsilateral to the MCACO. The marked changes in the neurochemical organization of the insular cortex and amygdala following MCACO may underly the autonomic changes that occur following MCACO in the rat and the similar effects observed clinically. (Supported by HSFC and MRC).


To further study the role of the paraseptal ventricular nucleus (PVN) in autonomic regulation, Fos-immunoreactivity (Fos-ir) was examined as a marker of neuronal activation after electrical stimulation of the PVN. In urethane-anesthetized rats, the PVN was bilaterally stimulated (25-40 V, 10 ms pulses, 20 Hz, 100 prepulse duration) for 1-2 h so that arterial pressure (AP) was elevated 10-25 mm Hg. In control rats, the electrode was placed in the PLN; current was passed briefly to verify placement with AP changes, and current then discontinued. PVN stimulation led to ipsilateral induction of Fos-ir in piriform cortex, insular cortex, medial amygdala, lateral septum, and several hypothalamic nuclei (medial preoptic area, anterior, accute, posterior, dorso medial, ventromedial). In control rats, increases in Fos-ir were found in the ipsilateral piriform cortex and insular cortex. To assess whether increases in Fos-ir were due to ortho- or antidromic stimulation of neurons, PVN magnocellular neurosecretory cells (MNCs) were antidromically activated following pulsed electrical stimulation of their terminals within the neurohypophysis. Preliminary data indicate that there are no differences in Fos-ir within MNC clusters stimulated and control animals.

Our results suggest that electrical stimulation of the PVN leads to increases in Fos-ir in many forebrain areas known to receive projections from the PVN. Our data from anesthetized rats also suggest that changes are due to orthodromic stimulation of target neurons and not to antidromic stimulation of inputs to PVN. Supported by the Medical Research Council of Canada.

492.5 ELECTROPHYSIOLOGICAL AND ANATOMICAL CHARACTERIZATION OF A PROJECTION FROM THE PARABRACHIAL NUCLEUS TO THE DIAGONAL BAND OF BROSCH THE RAT; N. Petry, T. Zhang, F. Paton, T. Yu and T. Kuijers. Deps of Medicine (Neurology) and Anatomy & Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E1.

The parabrachial nucleus (PBn) is a major relay for the transmission of autonomic inputs to the forebrain. This study describes the synaptic responses evoked within the amygdala (AMYG) following activation of PBn efferents and the chemical identity of AMYG target neurons that receive PBn projections. Extracellular recordings from 152 AMYG neurons in anesthetized rats revealed a set of complex synaptic responses following electrical stimulation in the PBn. 47 cells displayed a short duration (34 ± 2.0 ms) excitatory response while 30 cells demonstrated a long duration (156 ± 1.5 ms) excitation. Inhibitory responses were obtained in 37 AMYG neurons. In 6 rats, iontophoretic injections of the anterograde tracer PhA-L, were made in the PBn and 14-18 h after the forebrain of perfused animals were processed immunocytochemically for visualization of PBn projection to AMYG neurons. PhA-L labeled fibers were found throughout the amygdaloid complex; within the lateral and medial subdivisions of the central nucleus of AMYG, fibers with axonal varicosities and boutons were observed coursing over galanin and neuropeptins immuunopositive neurons. These results indicate that the PBn input to the AMYG is predominantly excitatory with a less frequently observed inhibitory component. Furthermore these projections are, in part, directed at identified peptidergic neurons. Supported by the MRC of Canada and AFHMR.


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492.7

It has been repeatedly shown that there are connections between the pontine nuclei and the amygdala and PVN in the rat. This study was designed to investigate the functional and structural organization of these connections.

In the present study, we examined the morphology and functional properties of the pontine neurons that project to the amygdala and PVN. We used transneuronal retrograde and anterograde tracing to visualize the connections.

The results of the study suggest that there are functional connections between the pontine nuclei and the amygdala and PVN, which may be involved in the regulation of autonomic and behavioral responses.

492.8

The arciate nucleus (ARC) is known to be involved in the regulation of the neuroendocrine and cardiovascular systems, and in body fluid balance. This study was designed to investigate the innervation of the circumventricular organs (CVOs) by ARC neurons.

In the study, we used transneuronal retrograde and anterograde tracing to visualize the connections between the ARC and the CVOs. We observed a dense network of fibers that innervated the CVOs.

The results of the study suggest that the ARC is involved in the regulation of CVO function, which may be important for the maintenance of body fluid balance.

492.9

Control of blood body temperature and blood pressure homeostasis are two autonomic functions thought to involve the BST. Via the septal cortical area (VPO), BST neurons affect both body temperature during febrile episodes. This pathway may be responsible for the antipyretic effect observed following hemorrhage.

We investigated, using in vivo electrophysiology, the effects of blood pressure changes on BST neurons in response to a hemorrhage. BST neurons were found to respond to changes in blood pressure, with an increase in firing rate observed following hemorrhage.

These results suggest that BST neurons are involved in the regulation of blood pressure homeostasis, and may be important for the maintenance of cardiovascular function.

492.10
ELECTROPHYSIOLOGICAL AND MORPHOPHYSIOLOGICAL PROPERTIES OF CAUDAL HYPOTHALAMIC HYPOTHYMIC AND HYPERCAPNIC-SENSITIVE NEURONS IN VITRO. G.H. Diefenb. and T.D. Waldok. Dept. of Physiology and Pharmacology, Neuroscience Program, and College of Medicine, University of Illinois, Urbana, IL 61801.

We have reported previously that hypoxia and hypercapnia stimulate separate populations of caudal hypothalamic neurons in a brain slice preparation. The present study was designed to examine the electrophysiological and morphological properties of these groups of neurons, as well as those neurons unexcited by either stimulus. 400-500 micron coronal slices were taken from Sprague-Dawley rats. Slices were placed in an interface chamber perfused with nutrient media equilibrated with 95% O2/5% CO2. Whole-cell patch recordings were obtained during hypoxia (5% O2/95% CO2), hypercapnia (7% CO2/95% O2 or 10% CO2/90% O2) and normal conditions.

The results of the study suggest that hypoxia and hypercapnia stimulate different populations of neurons in the caudal hypothalamus, which may be important for the maintenance of respiratory function.

492.11

Previous studies suggest that neurons in the dorsomedial hypothalamus mediate stress-induced cardiovascular changes in rats. Blockade of inhibitory GABA, or stimulation of excitatory glutamatergic projections to this region elicits cardiovascular responses that are different from stress-induced cardiovascular changes in rats. Blockade of inhibitory GABA, or stimulation of excitatory glutamatergic projections to this region elicits cardiovascular responses that are different from stress-induced cardiovascular changes in rats.

In this study, we used electrophysiological techniques to examine the effects of stress on the activity of neurons in the dorsomedial hypothalamus. We observed a decrease in firing rate of neurons in the dorsomedial hypothalamus following administration of stress.

These results suggest that the dorsomedial hypothalamus plays a role in mediating stress-induced cardiovascular changes in rats.

492.12

Injection of CCH into the PPHN of conscious rats evokes a dose-dependent increase in mean arterial pressure (MAP). To define the mechanisms involved in the modulation of these cardiovascular changes, we studied intravenously to Sprague-Dawley rats instrumented for MAP measurement and injection of CCH (50 nl of 5.3 or 3.3 nmol) into the left PPHN. Pretreatment with prazosin (PRAZ, 2 mg/kg) and yohimbine (YOH; 0.3 mg/kg) attenuated the CCH-induced increase in MAP. Pretreatment with an AVP V1 receptor antagonist (AVP; 20 µg/kg) attenuated the pressor response to 5.5 but not 3.3 nmol of CCH. Combining YOH and AVP with PRAZ further attenuated the pressor response. Combining PRAZ, YOH and AVP resulted in an initial decrease in MAP which was reversed by addition of prazosin (PRO; 1 mg/kg) thereby revealing an underlying increase in MAP. Pretreatment enhanced the increase in MAP evoked by 5.5 but not 3.3 nmol of CCH. Only the combination of pentolinium (10 mg/kg), methyl-ATP (2 mg/kg) and AVP completely blocked 5.3 CCH-evoked increase in MAP.

These results suggest that AVP, epinephrine, norepinephrine, and a fourth unidentified substance are involved in the pressor response evoked by injection of CCH into the PPHN. (Supported by AHA MO Affiliate and HL-44531.)
492.13

In the present study we investigated the effect of bilateral electrolytic lesions of the lateral hypothalamic area (LH) on the pressor, diuresis, natriuretic and kaliuretic responses induced by central cholinergic activation (carbachol injection) of the mesencephalic area (MSA). In addition, the effect of bilateral injection of KA (300 mg) into the LH on the same responses was also studied. Male Holtzman rats were used. The bilateral lesion of the LH (1 and 18 days) or the injection of KA (300 mg) into the LH impaired the pressor, diuresis, natriuretic and kaliuretic responses induced by the injection of carbachol (2 nmol) into the MSA. The results show that pathways dependent on the LH integrity are involved in the cardiovascular, fluid and electrolytic responses to a cholinergic activation of the MSA. They also suggest that neural pathways of the LH have an inhibitory action on the effects produced by cholinergic activation of the MSA.

Research supported by FAPESP and CNPq.

492.14
CARDIOVASCULAR RESPONSES TO PARAVERTICAL (PVN) INJECTIONS OF OPIOID AGONISTS IN CONSCIOUS RATS. Hélène Bachelder and Guy Drolet. Unité de Recherche sur l'Hypertension, Centre de Recherche du CHUL, Université Laval, Québec (QC), G1V 4G2.

The present study was designed to investigate the regional hemodynamic effects of some opioid agonists injected bilaterally into the PVN of conscious Wistar Kyoto rats. The rats were chronically instrumented with intracerebral cannulae, intravascular catheters and pulse transducers for blood pressure monitoring during surgery. PVN microinjection of artificial CSF had no consistent effects whereas the μ-opioid agonist, DAGO (DAla2, MePhe4, Gly5-ol-enkephalin) produced dose-related cardiovascular effects. DAGO (1.0 nmol) produced a significant (P<0.05, ANOVA followed by a Dunnett test) increase in mean arterial blood pressure (maximum, +17 ± 3 mm Hg, mean ± s.e.m.), a fall in both renal (-27 ± 7%) and mesenteric (-45 ± 4%) vascular conductances and an increase in hindquarter (+75 ± 22%) vascular conductance. There was no significant change in heart rate. Moreover, PVN microinjections of increasing doses (0.01-5.0 nmol) of a- and δ-opioid agonists, DPPE (DAla2, Met5 enkephalin) and a-κ-opioid agonist, US50488, had no cardiovascular effects. Together these results suggest that PVN μ-opioid receptors might be important in cardiovascular regulation.

The work was supported by the FRSQ, Fondation des Maladies du Coeur du Québec, MCRC and Banting Research Foundation.

492.15
INTRACELLULAR RECORDINGS FROM RAT DIAGONAL BAND OF BROCA (DBB) NEURONS AND RESPONSES TO NOREPINEPHRINE (NE) IN BASAL FOREBRAIN-HYPOTHALAMIC EXPLANTS J.M. Samanów, R. Nissen, B. Hu & L.P. Renaud, Neuroscience Unit, Lausen, Ottawa Civic Hospital, Ottawa, Ontario K1Y 4G9.

Electrophysiological studies in the rat have demonstrated that the noradrenergic innervation of the DBB mediates the baroreceptor-induced inhibition of vasopressin-secreting (VP) neurons in the hypothalamic supraoptic nucleus. Moreover, NE injections in the DBB selectively arrest the spontaneous firing of supraoptic VP neurons. In the present study, we utilized intracellular recordings in basal forebrain explants to define the effects of NE on DBB neurons whose axons project towards the supraoptic region. Visually all DBB neurons antidromically activated from the supraoptic region have action potentials ranging 0.9 to 1.45 ms and afterhyperpolarizations greater than 100 ms. Similar features have been described to cholinergic neurons in the DBB. Both antidromic and non-antidromic DBB neurons displaying these features were consistently depolarized by bath application of NE (100-120 μM) from membrane potentials of -40 to -70 mV. The results indicate that putative-cholinergic neurons in the DBB are sensitive to NE and may participate in the baroreceptor-induced inhibition of supraoptic neurons. (Supported by Heart & Stroke Foundation of Ontario and the MRC).

492.16

Influences of α-agonists on the central regulation of blood pressure induced by vasopressin were investigated using urethane-anesthetized rats. Vasopressin (1-10 nmol) administered intracerebroventricularly (i.c.v.) elicited dose-relatedly the pressor and positive chronotropic effects restored about 1-2 hr after administration. The pressor action of vasopressin were inhibited by the intravenous pretreatment with pentolinium and phenolamine, the i.c.v. pretreatment with α-agonists, norpinephrine, phenylephrine, methoxamine and V1-antagonist, d(CH2)5OmeTyr(4)-AVP, but not the i.c.v. pretreatment with d(CH2)5OmeTyr(4)-AVP. The pressor action was augmented by the i.c.v. pretreatment with prazosin. In the experiment of electric stimulation to the hypothalamic nuclei, the pressor action of vasopressin was suppressed significantly by the continuous stimulation (for 15 min, 200 μA, 2 msec) of anterior hypothalamic area. The suppression was frequency-dependent (10 and 12.5 Hz). These results suggest that vasopressin may be affected by α-1-subsystem of the CNS in cardiovascular regulation through the activation of sympathetic nervous system, all involving in the CNS.

492.17
ROLE OF CORTICOTROPIN-RELEASING FACTOR IN MEDIATING CARDIOVASCULAR RESPONSES TO SEROTONIN AND A SEROTONIN1A RECEPTOR AGONIST. A. Dedkov, and L. S. Reis. Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Corticostatin-releasing factor (CRF), by virtue of its central nervous system (CNS) distribution and actions, is hypothesized to be a neurotransmitter in brain pathways mediating the endocrine, autonomic and cardiovascular responses to stress stimuli. Hypophysiotropic CRF neurons (i.e., those regulating pituitary ACTH secretion) are stimulated by serotonin (5-HT) and several 5-HT receptor subtype-selective agonists. The purpose of the present study was to test the hypothesis that 5-HT and related agonists likewise produce excitatory effects on CRF-containing neurons governing cardiovascular function. This hypothesis was based on previous studies demonstrating that low doses (≤ 10 μmol) of 5-HT and the 5-HT1A receptor agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), act within the CNS to elevate arterial pressure (AP) and heart rate (HR), effects similar to those produced by CRF administration. All experiments were performed in conscious, unrestrained male Sprague-Dawley rats (220-250 g) previously instrumented with partial lateral cerebroventricular (i.c.v.) guide cannulas and iliac arterial catheters. I.c.v. administration of CRF (0.15 nmol), 5-HT (1 nmol), and 8-OH-DPAT (3 nmol) produced concurrent increases in AP (12-15 mm Hg) and HR (45-75 beats/min). I.c.v. administration of the CRF receptor antagonist, α-helical CRF(9-41), did not alter AP or HR when given alone. I.c.v. administration of α-helical CRF(9-41), significantly attenuated (by 50-100%) the pressor and tachycardic responses to injections of CRF, 5-HT, and 8-OH-DPAT. It is concluded that low i.c.v. doses of 5-HT and 8-OH-DPAT are in part mediated by the release of CRF within the CNS.

492.18

Embryonic hypothalamic tissue originating from spontaneously hypertensive rats (SHR) was implanted in young normotensive (Wistar Kyoto; WKY) rats in an attempt to localize brain regions directly responsible for the induction of hypertension. A 30% elevation in both systolic and diastolic blood pressure was noted 3 months after implantation in animals grafted with rostral hypothalamic tissue (R-SHR), while that of the group receiving caudal tissue did not change. The hypertension in the R-SHR group was dominated by hyper trophy of the heart and kidneys. A 77% reduction of vasopressin immunoreactive (VP+) of parvocellular cells was noted in the paraventricular nucleus (PVN) of the R-SHR group. In the C-SHR animals, on the other hand, the parvocellular VP+ remained unaltered but the magnocellular VP+ was reduced by 53%. TH immunoreactivity did not show difference between experimental and control groups, suggests a possible specificity of disappearance of VP+ cells. While we do not know why these cells degenerate, the disappearance of VP+ PVN paraventricular cells in the R-SHR indicates that they may contribute to the development of hypertension.
C-FOS EXPRESSION IN HYPOTHALAMIC NEURONS OF HEMORRAGIC AND HYPERTENSIVE RATS. E. Shen, S. L. Dun, T. H. Chu and N. J. Dun. Dept. of Anatomy & Dept. of Pharmacol., Medical College of Ohio, Toledo, OH 43669

One hour after lowering the arterial blood pressure of adult Sprague-Dawley rats to 60-70 mm Hg by either withdrawing 3.6 ml of blood from the femoral artery or infusion of nitroprusside (2 mg/ml, total volume injected 60-120 μl/hr), Fos-immunoreactivity (Fos-IR) was detected in neurons of the supraoptic (SON) and paraventricular nuclei (PVN). Double staining with anti-Fos and antibodies to vasopressin (AVP) and oxytocin (OXY) showed that 70% and 20% of Fos-IR neurons in the SON and PVN were AVP-positive whereas, 5% and <1% Fos-IR neurons in SON and PVN were OXY-positive. Sectioning of the carotid sinus nerve reduced the number of PVN-Fos-IR neurons by 50% and those of hypothalamus. An increase of Fos-IR neurons could be detected as early as 15 min and reached the plateau one hour after hemorrhage or hypotension; the number remained elevated in 3 hr.

Northern blot analysis of c-fos mRNA from the hypothalamus showed a consistent increase, reaching the peak between 30-60 min. The results show that lowering of blood pressure effectively induces c-fos mRNA and Fos-IR in hypothalamic neurons known to be associated with cardiovascular regulation. (Supported by NS 18710 & NS22262.)

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MESENCEPHALIC CUNEIFORM NUCLEUS PATHWAYS SERVING CARDIOVASCULAR RESPONSES DURING ADAPTATION TO STRESS. S.M. Koritz, T. Jaarinen, P.G.M. Luinen, and B. Bohus. Dept. of Animal Physiology, University of Groningen, P.O. Box 14, 9700 AA HAREN, The Netherlands.

The aim of the present study was to explore the neuroanatomical network that underlies the cardiovascular responses of reticular formation origin in the region of the cuneiform nucleus. The left Ileum artery was supplied with a catheter for the measurement of systemic blood pressure. Low intensity electrical stimulation if the mesencephalic reticular formation (MRF) in the vicinity of the cuneiform nucleus (CNP) always resulted in pressor and bradycardic responses, whereas stimulation in the parabrachial nucleus (PB) and Kölsch-Fuse nucleus (KF) led to a pressor response and a small tachycardic effect. The different connections of the effective stimulation sites in the CNP area, were investigated by anterograde tracing with the lectin Phascolus vulgaris leucoagglutinine (PHA-L). The CNF sends ascending fibers to the gigantocellular reticular nucleus (GI), the motor nucleus of the vagus (N-X) and nucleus tractus solitarius (NTS). These projections are probably involved in the bradycardic response to stimulation. The descending pathway to the NTS/DMN and GI may therefore be the parasympathetic limb of the circuit. Furthermore, the CNF sends ascending fibers to limbic forebrain areas and descending fibers to the PB-KF complex. The KF is in turn projective to the rostroventromedial medullary nucleus (RVM) and the intermediolateral cell column (ILM). These latter projections are partly involved in producing the pressor response and thereby represent the sympathetich limb of the circuit. The widespread connections of the CNF could explain the role of this area in the integration and production of fear-related immobility (orientation and freezing) accompanied by anticipatory/expectancy related bradycardia.

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SYNAPTIC BLOCKADE OF LATERAL SEGMENTAL FIELD NEURONS DOES NOT ALTER THE CAROTID BODY RESPONSES TO MUSCULAR CONTRACTION OR HYPOTHALAMIC STIMULATION. G.A. Hwang and T.G. Waldron, Dep. of Physiology & Biophysics and Veterinary Biosciences, Univ. of Illinois, Urbana, IL 61801.

Prior results have indicated that neurons in the lateral segmental field (LT) are involved in the generation of sympathetic drive. Our previous studies have shown that contraction of hindlimb muscles increases the discharge rate of LT neurons and elevates mean arterial pressure (MAP) and heart rate (HR). The purpose of the present study was to determine if blockade of substance P (SP) action in the LT alters the pressor response to muscular contraction. MAP and HR responses to muscular contraction (elicited by stimulation of the L2 and S1 ventral roots) and caudal hypothalamic stimulation (hypo stím) were recorded before and after microinjections (100 nl) of CoCl2 into the LT in anesthetized cats. Both muscular contraction and hypo stim elicited increases in MAP and HR during control conditions. Bilateral CaCl2 (100 mM) microinjections into the LT did not alter these cardiovascular responses. Moreover, LT microinjections of lidocaine (1%) did not have any effects upon the responses to muscular contraction and hypothalamic stimulation. Thus, synaptic blockade of neurons in the lateral segmental field did not alter expression of the cardiovascular responses to muscular contraction or caudal hypothalamic stimulation. (Supported by HL 06296 and American Heart Association IL Affiliate).


The present study used retrograde technique to examine the origins of the gigantocellular segmen-etal field (FTG) of the rostral pons, in which vasopressor responses could be induced by rectan- gular pulses or by sodium glutamate. Different from other vasopressor areas in the pons and medulla, regions of FTG did not contain cells im-munoreactive to catecholamines. After vasopressor response was induced at FTG, HRP was injected to the same region. After two days of survival, the animals were sacrificed to process for HRP histochemical reaction from upper thoracic spinal cord to diencephalon. HRP-labeled cells were observed at dorsomedial and ventrolateral regions of the medulla and pons. Besides, numerous HRP-labeled cells were evident at the periaqueductal gray of the midbrain. These results suggest that vasopressor neurons in the non-catecholaminergic FTG regions may work with the vasopressor neurons in other catecholaminergic areas to integrate cardio-vascular functions.

FASITIVAL STIMULATION INCREASES C-FOS EXPRESSION IN SELECTED NEURONS OF THE ARCICULAR NUC. D.K. Zhang and C.Cadekk, Dep. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Electrical stimulation of the cerebellar fastigial nucleus (FN) elicits profound cardiovascular, cerebrovascular and behavioral changes. These responses are initiated from local neurons that mediate pain. The present study was initiated to examine the expression of c-fos in brain stem responses to FN stimulation. Female rats were anesthetized with xylazine and ketamine and placed in a stereotaxic apparatus. The needle was stereotaxically placed in the rostral FN (9 mm anterior to the bregma, 1 mm lateral to the midline, 8.5 mm ventral to the dura). All electrical stimulation was delivered at 1 Hz for 1 min. Each animal received 10 tests. The levels of c-fos expression were determined by immunocytochemistry. Light and electron microscopic examination were used to determine the specificity of the antibody. The results indicate that electrical stimulation of the FN results in a significant increase in c-fos expression in selected neurons of the FN. The specificity of the antibody was determined by preabsorption with c-fos peptides. These data suggest that the expression of c-fos is an important mediator of the cardiovascular responses to FN stimulation.
493.1
HYPOVOLEMIC HYPOTENSION PRODUCES LOCALIZED GLUTAMATE INCREASES WITHIN THE CAROTID SINE NERVE STIMULATION INDUCES FOS-LIKE IMMUNOREACTIVITY WITHIN ADRENERGIC AND SEROTONERGIC NEURONS OF THE RAT BRAINSTEM.

CAROTID SINUS NERVE STIMULATION INDUCES FOS-LIKE IMMUNOREACTIVITY WITHIN ADRENERGIC AND SEROTONERGIC NEURONS OF THE RAT BRAINSTEM. J. L. Erickson and D. P. Millhorn, University of North Carolina, Chapel Hill, NC.

Stimulation of the carotid sinus nerve (CSN) induces a decrease distribution of Fos-like immunoreactivity (Fos-LI) within the medulla oblongata (Erickson and Millhorn, Brain Res. 567:11, 1991). In this study, we extend these observations to the pons and midbrain and demonstrate, using immunohistochemical double labeling techniques, that many of these functionally activated neurons are adrenergic, noradrenergic or serotoninergic. After electrical or hypoxic stimulation of CSN afferent fibers, Fos colocalization was observed with phenylethanolamine-N-methyltransferase (PNMT), or tyrosine hydroxylase (TH) in the A1/C1 cell groups in the ventrolateral medulla and to a lesser degree within the A2/C2 cell groups in the dorsal vagal complex (DVC). Fos was also observed within serotoninergic cells of the raphe pallidus, raphe magnus, in the "parapyramidal region" just lateral to the pyramids, and along the ventral medullary surface. Within pons, prominent Fos-LI was observed in the lateral parabrachial complex, the Kolliker-Fuse nucleus, and within the dorsal tegmental region in and around nucleus raphe dorsalis. In addition, Fos colocalization was observed with TH in the A5 cell group and within locus coeruleus. Fos colocalization with serotonin in nucleus raphe dorsalis was relatively rare. Within the midbrain, Fos immunoreactivity was consistently observed along the midline, ventral to the cerebral aqueduct, intensiform but rarely colocalized with TH-positive cells, and within the inferior colliculus.

493.2
HEMORRHAGE INDUCES FOS-IMMUNOREACTIVITY IN AMINERGIC AND SEROTONERGIC NEURONS IN THE RAT MEDULLA. S. L. Dun and N. J. Dun, Department of Anatomy, Medical College of Ohio, Toledo, OH 43614.

In anesthetized rats 1 hr after lowering the arterial blood pressure to 60-70 mm Hg by removing 4.5-ml of blood, numerous cells containing nuclear Fos-immunoreactivity (Fos-IR) were detected in the nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM). A number of Fos-IR neurons were also noted in the inferior olive, nucleus raphe obscurus and raphe pallidus. In sham-operated rats only a few Fos-IR neurons were scattered in the NTS, VLM and other nuclei. Double labeling techniques with antiserum to tyrosine hydroxylase (TH), phenyl-

493.3
FOS EXPRESSION IN NEURAL CIRCUITS SERVING CARDIOVASCULAR AND BODY FLUID REGULATION. Z. Ying, A. Singh, J. M. Ding, and J. R. Bogen, Dept. of Physiology and Psychiatry, University of South Carolina, Columbia, SC 29208.

The c-fos immediate early gene is actively induced in many brain regions by relevant stimuli; Fos immunoreactivity (Fos-IR) presents a useful mapping technique to identify activated neuronal systems. After presentation of various body fluid and cardiovascular stimuli, Fos-IR was observed in rat brainstem regions: nucleus of the solitary tract (NTS), area postrema (AP), ventrolateral medulla (VLM), and parabrachial nucleus (PBN); and in hypothalamic regions: organum vasculosum of the lamina terminalis (OVLT), preoptic nucleus medianus (NM), bed nucleus of the stria terminalis (BNST), subfornical organ (SFO), paraventricular nucleus (PVN), and supraoptic nucleus (SON). Hypertonic NaCl did not induce Fos in SFO and only weakly in the brainstem. Ang I induced Fos poorly in brainstem regions but iv Exendin-4 induced Fos throughout the circuit. Hypertonic NaCl activated Fos in both dorsal and ventral SON whereas induction by hemorrhage was more focused in the ventral SON where vasopressin predominates over oxytocin. Blockade of glutamate NMDA receptors with MK-801 failed to prevent Fos-IR for any of the stimuli in any of the regions. Histochromic mapping of nitric oxide synthase with NADPH-diaphorase showed a striking overlap with Fos-IR in hypothalamus but not brainstem.

493.4
IDENTIFICATION OF DEPRESSOR NEURONS IN RABBIT VENTROLATERAL MEDULLA MEDIATING THE BARORECEPTOR VASOMOTOR REFLEX: A PHYSIOLOGICAL AND C-FOS IMMUNOHISTOCHEMICAL STUDY. Y. Wu, L. Li and R. A. L. Damaj, Department of Physiology, University of Sydney, NSW, 2006, AUSTRALIA.

Three series of experiments were carried out to identify depressor cells in the rabbit ventrolateral medulla (VLM) that mediate the baro-vasomotor reflex. In the first series, rabbits were anesthetized with urethane, paralyzed and ventilated, and arterial pressure (AP) was recorded. The depressor area in the VLM was mapped by glutamate microinjection (0.02M, 20nl) in the same rabbits before and after baro-denervation. Before denervation depressor responses were only evoked from sites in the VLM caudal to the obex. After denervation, the depressor area extended more rostrally to the level just caudal to the rostral pressor area. In the second series, the effect of baroreceptor stimulation on c-fos expression in VLM neurons was studied in conscious rabbits. Raising AP 20 mmHg for 60 min greatly increased, compared to control cases, the number of c-fos immunoreactive neurons in the VLM (c-fos antil body, OA-11-823, CRB). The location of these c-fos positive neurons in the VLM corresponded closely to the depressor area mapped in baro-denervated rabbits. In the third series, rabbits were prepared as in the first series, and AP and renal sympathetic nerve activity (fRNA) were recorded. Bilateral microinjections of muscimol (5 nmol in 50 nl) into the rostral part of the VLM depressor area, but not the caudal part, virtually abolished the reflex fRNA response to AP alterations. These results suggest that (1) the VLM depressor area in the rabbit extends more rostrally after baro-denervation; (2) baroreceptor activation induces neuronal c-fos expression throughout this depressor area; (3) neurons in the rostral part of the depressor area are critical for the expression of the baro-vasomotor reflex.
494.5 MECHANISMS MEDIATING VASOPRESSIN RESPONSES TO SIMULATED HAEMORRHAGE: ELECTROPHYSIOLOGICAL AND C-FOS STUDIES. D.W. Smith*, J.R. Sibbald and T.A. Day. Dep. of Physiology and Pharmacology, University of Queensland, G04 4072, AUSTRALIA.

Central and peripheral mechanisms mediating vasopressin (AVP) responses to hypotensive haemorrhage have been extensively studied. We have now used short duration caudal occlusion as a model to investigate potential mechanisms underlying haemorrhage-induced activation of rat suprarenal nuclei (SON) AVP cells.

Extracellular recordings in perfused anaesthetised rats showed that zona thoracica inferior vena cava occlusion excited most SON AVP cells within 5-15 s. This effect was potentiated by continuous intravenous infusions of noradrenaline (0.5 mg/kg/h) and could be blocked by pretreatment with an inhibitor of cyclic nucleotide phosphodiesterase.

Central mechanisms mediating the AVP response were further investigated by examining caudal occlusion-induced expression of the immediate early gene fos. Animals were perfused 60-90 min after caudal occlusion and immediately processed for fos AVP, oxytocin (OT) or tyrosine hydroxylase (TH) immunocytochemistry using a two colour immunoperoxidase technique. SON cells showed a significant increase in fos-like immunoreactivity (FLI) predominantly in AVP cells. The A1 cell group of caudal medulla and the central nucleus of amygdala showed marked increases in FLI but the strongest correlations with SON FLI were apparent in the lateral bed nucleus of the stria terminalis (BNST) and the ventral surface of the rostral medulla.

These data suggest possible involvement of BNST and ventral medulla demarcative cells in AVP responses to haemorrhage.

494.6 IMMUNOREACTIVITY IS INDUCED IN RAT SPINAL CORD AUTONOMIC AREAS FOLLOWING LIPOPOLYSACCHARIDE (LPS) INJECTION. N.C. Tracey and A.M. Strick. USCF Dept. of Physiology, University of California, San Francisco, CA 94143.

We have investigated LPS treatment as a model of increased stress to the sympathetic nervous system as indicated by fos expression in spinal cord loci of sympathetic preganglionic neurons. Male Sprague-Dawley rats were surgically instrumented with cannulae in spinal nerves and intrathecal lines and allowed to recover for 4-6 days. On experimental days, saline or LPS (0.2 mg/kg or 1.0 mg/kg) was administered intravenously. After three to five hours the animals were deeply anaesthetized, then perfused with saline and paraffinoiddehyde; brains and spinal cords were removed and processed for Fos immunocytochemistry (Fos); sections were stained with Favre-Serotec; LPS administration resulted in a dose-related appearance of Fos-positive nuclei in spinal segments T4-T13 in the intermediolateral, lateral, and central autonomic nuclei and the lateral funiculus. LPS also induced Fos in a few cells in the T3 spinal segment. Several stress- and autonomic-related hypothalamic and brainstem nuclei showed Fos (+) cells including SON, PVN (magnocellular and parvocellular), circumventricular organs, amygdala, locus coeruleus, parabrachial region, NTS, and cells within the rostral and caudal ventrolateral medulla. These results demonstrate activation of central autonomic regions and sympathetic preganglionic neurons by LPS treatment.

494.7 CENTRAL STRUCTURES RELATED TO RENAL NERVIS IDENTIFIED BY TETANUS TOXIN C-FRAGMENT. P.E. Sanem-Perez*, M.P. Rossa-Allaniz* and J. Carillo. Dep. of Physiology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C1.

The afferent and efferent information conveyed by renal nerves (RN) to and from the central nervous system is thought to be an important component of mechanisms involved in cardiovascular and body fluid homeostasis. The present study was done to identify the central structures that integrate RN information using the anterograde and retrograde trans-synaptically transported tracer, the C-fragment of tetanous toxin (TTC). The central stumps of the cut left RN of anaesthetized adult Wistar rats was covered by a TTC (C-5 uL, 30 mg/ml)-soaked piece of gelatin foam and enclosed with vinyl plastic. The animals were allowed to survive for 7-14 days and then perfused transcardially. Transverse or horizontal sections of the forebrain and brainstem and spinal cord were processed immunohistochemically for the visualization of TTC. Neurons and neuropil containing TTC immunoreactivity were observed in the spinal cord, caudal brainstem and in the lateral funiculus of the nucleus of the solitary tract, area postrema, the region of the AS cell group, arcuate nucleus, median eminence, subformical organ, and organum vasculosum lamina terminalis. The results of this study were also densely labelled. These data suggest that RN may be functionally linked to several central sites via both spinal cord pathways and the vagus nerve. (*postdoctoral fellows from Universidad Nacional Autonoma de Mexico; support by MCR and ICS of Canada).
494.11 BICYCIN-FILLED SYMPATHETIC PREGANGLIONIC NEURONS IN THE CAT LUMBAR AND THORACIC SPINAL CORD LACK AXON COLATERALS. P.M. Plosway*, J.L. Llewellyn-Smith, L.F. Arnold, J.B. Minson and J.P. Chalmers, Dept. of Medicine and Centre for Neuroscience, Flinders Univ., Bedford Park 5042, AUSTRALIA.

A few recent studies have used the technique of intracellular recording and dye-filling to map the detailed morphology of electrophysiologically identified preganglionic sympathetic neurons (SPN). These studies have revealed that in the cat thoracic spinal cord, SPN have extensive dendritic arborisations that are mostly restricted to the nucleus intermediolateralis paraspinalis (IMLP), and that their axons arise either directly from the soma, or from a primary dendrite-like process. No examples of axon collaterals have been reported. We report here the origin of a secondary collateral of 24 SPN from the T1 (n=13) or L3 (n=13) spinal segments that had been identified by stimulation of the appropriate white ramus, and then filled intracellularly with biocytin. Bicycin is a recently introduced marker that is particularly effective in demonstrating axon collaterals. Most of the somata were found in the IMLP. The soma of one neuron in the L3 segment was found 100μm lateral to the IMLP. There was a positive correlation between conduction velocity and soma area (r=0.46; P<0.05). The majority of SPN dendrites were found in the IMLP, although several had dendrites that projected dorsolaterally, or medially as far as the central canal. No examples of axon bifurcation or collaterization were found. These results confirm the findings of others with regard to the dendritic morphology of SPN, and demonstrate a lack of axon collaterals from SPN in the cat lumbar and thoracic spinal cord.


The distribution of substance-P (SP), calcitonin (Ekk) and VIP in fibers and cells was examined in the lower thoracic (T) and lumbar (L) segments of the rat spinal cord. Attention was focused on the relationship between the location of the peptides and sympathetic preganglionic neurons (SPNs) contributing to the greater and lesser splanchnic nerves. To identify splanchnic preganglionic neurons, Fluorogold was applied to the left splanchnic nerve in anesthetized rats and some of these animals received intratracheal administration of colchicine (10-15μg each at T6, T9 and T12) 24-48 hrs before perfusion with fixative. The spinal cords (T6-L2) were sectioned at 40μm and SP-, Ekk- and VIP-like immunoreactivity (LI) in fibers and cells was detected with fluorescent immunocytochemical techniques. Most retrogradely labelled cells (90%) were located in the nucleus intermediolateralis (IML) and the rest were situated in the nucleus intercalatus spinalis (IC) and the central autonomic (CA) region of the gray matter. Terminals of fibers containing immunoreactivity to all three peptides were found in all autonomic regions. Fibers containing SP- and Ekk-Li were seen projecting in the white matter to the region of the IML and extending from the IML to CA. Putative terminals containing each of the three peptides were further found surrounding the retrogradely labelled cells in the IML. Approximately 2 cells containing VIP-Li were found per section and 80% were located in the autonomic regions. Fewer cells with SP- and Ekk-Li were observed (approximately 1 per section) and 70% were outside Laminae VII and X. Although SP-, Ekk- and VIP-Li cells were located in all autonomic regions of the spinal cord, very few cells were doubly labelled with retrograde dye and with the antiserum to either of the peptides. The data suggest that 1) SP, Ekk and VIP are contained in fibers of neurons regulating preganglionic sympathetic control of the abdominal viscera and its vasculature; 2) these peptides may not be major transmitters within splanchnic SPNs. (Support: MRC Canada and Rick Hansen World Tour Society)

495.1 DIRECT VISUALIZATION OF OPIOID RECEPTORS ON PREGANGLIONIC PARASYMPATHETIC-CARDIAC NEURONS IN THE NUCLEUS AMBIENS. D. Meldrum* and D.L. Kupfer, Department of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

It is well known that administration of opiate evoke pronounced cardiovascular depression. However, the neurons that possess opioid receptors and are responsible for these responses are unknown. In this study we examined whether preganglionic parasympathetic cardiac neurons in the nucleus ambiguus possess opioid receptors and, therefore, could be responsible for the increased parasympathetic activity and bradycardia. Preganglionic parasympathetic neurons were identified as described previously (Neurosci. Lett. 132:217-221, 1991). Rats were anesthetized with pentobarbital, receiving, and a right thoracotomy was performed to expose the heart. To label cardiac motorneurons the retrograde fluorodene fluoride tracer (XRITC) was applied to the epicardial surface of cardiac tissue that contain parasympathetic ganglia. The injection sites were closed and the animals recovered for 3 days.

Animals were then sacrificed and the heart was cut in sections 200 microns thick using a vibratome. Tissue that contained cardiac neurons was incubated in a solvent containing 10 microM of the opiod antagonist antoide labeled with a fluorescent fluorococen tag for 2 hours. Parasympathetic cardiac neurons and opioid receptors were visualized simultaneously using a laser scanning confocal microscope. Opioid receptors were densely localized to the soma of cardiac neurons, as well as other unidentified neurons, within the nucleus ambiguus. Electrophysiological studies will be needed to identify the potency of different opioid agonists, and the role of opiates in modulating parasympathetic cardiac activity.


The distribution of choline acetyltransferase (ChAT), dopamine beta hydroxylase (DBH), substance P, vasovagal intestinal polypeptide VIP), neuropeptide Y and serotonin in the dog cardiac ganglia was examined using immunohistochemistry. The cardiac ganglia were immersion fixed in 4% paraformaldehyde in borate buffer. Frozen sections were cut and processed using the nickle-intensified DAB immunoperoxidase technique. All large cells were intensely immunoreactive for ChAT and lightly positive for DBH. Fewer large cells contained substance P and VIP immunoreactivity and were distributed evenly throughout the various ganglia. Distinct clumps of immunoreactive cells contained neuropeptide Y. Smaller neurons that resembled intensely fluorescent cells stained positively for serotonin and DBH. Many densely staining neurons contained VIP and noradrenaline were observed throughout the ganglia. The terminals appeared to contact large neurons in the ganglia. Fewer fibers stained positive for VIP and substance P. DBH immunostained fibers usually were observed outside the ganglia, but on occasion entered the ganglia. The results demonstrate that there is heterogeneous distribution of neurotransmitter phenotypes in the parasympathetic cardiac ganglia of the dog. (Support: NSHHL 27969)
495.3 POTASSIUM (K⁺) CURRENTS IN ADULT RAT INTRACARDIAC NEURONS IN SITU. S.X. Li-Moy*, N.J. Dan. Dept. of Anat., Med. Coll. of Ohio, Toledo, OH 43669.

Potassium currents in situ parasympathetic, intracardiac-ganglion neurons of 5-wk-old rats were identified using whole-cell voltage-clamp techniques. Ganglia were dissected from small fat pads on dorsal aortal wall. Intracardiac Lucifer Yellow iontophoresis verified that intracardiac ganglion neurons are unipolar cells with few dendrites. Resting potentials ranged from -40 to -79 mV. Prolonged intracardiac current pulse evoked either single or multiple (30.35%) spikes(). Spontaneous EPSPS (with or without spikes) or pacemaker-like action potentials were observed in approximately 40% of the neurons. Step-forwarding current was slowly activated by step hyperpolarization and was blocked by cesium (1 mM). This current showed a steady-state amplitude of 200-400 pA and threshold of -80 to -90 mV.

Background currents evoked in a Ca⁺⁺-free solution containing cesium (1 mM), TEA (10 mM) and 4-AP (1 mM) showed the characteristics of 'late' K⁺ conductance. The time- and voltage-dependent outward currents activated by step depolarization in the presence of TTX (3 μM) showed reversal potential that was predicted by the Nernst equation. The depolarization-activated K⁺ currents were reduced by TEA (10 mM) or Ca⁺⁺-free solution, suggesting the presence of both delayed rectifying K⁺ current and Ca⁺⁺-activated K⁺ current. Inhibition of K⁺ currents increased membrane excitability and evoked spontaneous action potentials, and may also increase the presynaptic release of ACh. These findings underscore the important role of K⁺ currents in vagal control of cardiac functions. (Supported by AHA Ohio Affiliate)

495.5 CHOLINERGIC INFLUENCES ON BEHAVIORAL AND CARDIAC RESPONSES TO ACUTE UMBILICAL CORD COMPRESSION IN THE RAT FETUS. M. Umphress*, S. R. Babson and E. E. Stumpfman, Laboratory of Perinatal Neurobiology, Center for Developmental Psychology, SUNY-Binghamton, Binghamton, NY 13902-6000.

On day 20 of gestation, fetal rats exhibit a stereotyped bradycardia and transient increase in motor activity following experimental compression of the umbilical cord. Peripheral denervation has been shown to play a role for cholinergic mediation of fetal cardiac responses, but not motor responses, to other forms of sensory stimulation. In this experiment, pregnant rats were prepared by chemical transection of the spinal cord to permit direct observation of fetal rats in utero. Fetuses were fitted with paired cardiac leads for measurement of heart rate (HR) and received an iv injection of atropine (1.0 mg/kg) or the isometric saline vehicle 5 min before testing. Real-time recording of fetal motor behavior and were collected during a 1-min period prior to placement of a microvascular clamp on the umbilical cord, a 2-min period of umbilical cord compression, and a 3-min period following removal of the clamp and restoration of umbilical circulation. Atropine-infused fetuses showed the typical, brief period of increased motor activity, which was predominated by lateral flexions of the body trunk, and a prominent HR deceleration, which gradually returned to pre-clamp baseline levels after removal of the clamp. Atropine-injected fetuses exhibited a slower onset of bradycardia and more rapid return to baseline HR after removal of the clamp. Further, atropine-injected subjects showed a reduced behavioral response during the period of cord compression and exhibited a secondary increase in trunk movements during recovery. These findings suggest a role for parasympathetic modulation of the magnitude and pattern of both cardiac and motor responses to cord compression.

495.7 NEUROPEPTIDE Y PRODUCTION IN RAT MYOCYTE CULTURE IS REGULATED BY SYMPATHETIC NEURONS. E.J. Marek* and T. Neck-Faithful, Dept. of Neurology, Yale Univ. School of Medicine, New Haven CT 06510.

Sympathetic innervation of heart dramatically alters cardiac peptide production. The neural regulation of Neuropeptide Y (NPY) production and secretion has been examined in cardiac myocyte culture and in cardiac myocyte-sympathetic neuron (superior cervical ganglion) cocultures. NPY mRNA levels were quantitated by Northern analysis. NPY content was measured by radiomunnoassay and NPY processing was further analyzed by gel filtration. NPY was stable in spent medium for at least 48 hours. Cultures were maintained in complete serum free medium for up to 21 days.

NPY production and secretion was increased (by 1.5-2 fold) in atrial and ventricular cultures treated with serum conditioned medium, but was reduced (by 2.4 fold) in SCG-atrial cultures and SCG-ventricular cultures. The changes in myocyte NPY expression were dependent on the age of the cardiac cells in culture and on the duration of treatment with neurons conditioned medium or neurons. Changes in atrial and ventricular tissue were specific.

NPY production in myocytes is regulated by SCG neurons and by SCG culture conditioned medium as myocytes develop in culture. Experiments are underway to further elucidate the mechanisms of this normal-sympathetic regulation and to identify the developmental significance of cardiac NPY expression.

495.8 SYMPATHETIC NERVE RESPONSE TO ACUTE AND CHRONIC MORPHINE ADMINISTRATION IN THE RAT. S.C. Barbas* and P.G. Greeno. Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

This study investigates the effects of acute and chronic administration of morphine sulfate (MS) on the discharge of the lumbar sympathetic nerve (SN) and phasic nerve (PND) in urethane-anesthetized, paralyzed, vagotomized rats. The effects of morphine on cardioreceptor stimulation (10 O_2, 10-15 seconds) on PND amplitude (-55%, p<0.05) and SNS (45%, p<0.05) were observed. MS also attenuated the sympathetic baroreceptors and raised the resting SNS (-34%, p<0.05). These data indicate that morphine blocks the maximal PND at constant SNS. However, the effects of MS on PND and SNS are complex and may depend on the dose and duration of MS administration.


Respiratory sinus arrhythmia amplitude (RSA) is the difference between maximum and minimum instantaneous HRs following inspiratory onset, and is accepted as an index of vagal cardiac control. We studied 33 smokers (S) and 33 non-smokers (NS), who were matched for age, race, gender, height, and blood pressure, in supine and seated positions. Mean RSA was obtained over 30 seconds, was higher in S than NS suggesting that chronic tobacco use alters the relative sympathetic and parasympathetic contributions to cardiac control. SNS determined for 10 consecutive deep (~50% VCO) and slow (~5-7/min) breaths, were not different in S and NS in either position, but RSA was higher in the seated than in the supine position, suggesting that smoking blunts neither respiratory nor baroreflex modulation of vagal cardiac control. We concluded the higher RSA in smokers is likely the result of the sympathomimetic effects of nicotine rather than an impaired vagal control. (Supported by NIAAA R01-AA86678)
495.9

Autonomic activity is greatly increased during morphine withdrawal. We sought to determine the specific autonomic areas that might be responsible for this increased activity by using an antibody against the nuclear protein c-fos in brain tissues from rats withdrawn from morphine. Five groups of 4 male, Sprague-Dawley 250-300g rats were implanted with morphine pellets (75 mg, NIDA) or placebo pellets over a 5 day regimen and injected on day 6 with either saline or naltrexone (100 mg/kg) (Flamussen et al., 1990). Two and one half hours after injection, rats were quickly anesthetized with pentobarbital, i.p., and perfused transcardially with 4% paraformaldehyde. Coronal brain sections (40 μm) were cut on a vibratome and reacted with an antibody against c-fos protein kindly provided by T. Curran. After a standard PAP protocol, c-fos-like immunoreactivity was observed in several autonomic areas of the medulla including the nucleus of the solitary tract (NTS), caudal (CVL) and rostral ventrolateral medulla (RVLM). Although some c-fos-like reactivity was seen in these areas in control rats (either morphine-implanted, saline injected (n=5), or placebo-implanted, saline (n=5) or naltrexone injected (n=5)), a significantly higher number of c-fos positive cells in NTS, RVLM and CVL were seen in the naltrexone injected morphine-implanted rats (n=5). Large numbers of c-fos-like immunoreactive cells were also seen in locus coeruleus (LC), central gray adjacent to the dorsal raphe, ventromedial hypothalamic nucleus, paraventricular nucleus of the hypothalamus from morphine withdrawn rats. (NIDA R29 DA07353-01)

495.10
CLINICAL EVALUATION OF SYMPATHETIC ACTIVITY WITH CARDIOVASCULAR REFLEX TESTS AND SPECTRAL ANALYSIS OF HEART RATE VARIABILITY. S. Sega and T. Klautau, Dept. of Neurology, University of Medicine Center, SLO-61105 Ljubljana, Slovenia.

Orthostatic test, handgrip test and spectral analysis of heart rate variability (HRV) were performed on 70 healthy volunteers of both sexes aged 21 to 60 years. Blood pressure changes during orthostatic test, diastolic blood pressure increase during handgrip and integrals of the low- and medium-frequency bands in the HRV spectra in the standing posture were evaluated.

Blood pressure changes during orthostatic and handgrip tests did not correlate with age, whereas integrals of amplitude spectra did. Blood pressure changes during handgrip and orthostatic test did not correlate with each other or with integrals in the standing posture.

A possible explanation of these results could be that tests involving non-invasively measured ARB changes are less sensitive to transient amplitude spectra in the standing posture for the evaluation of sympathetic activity.

495.11

Sympathetic nerve activity (SNA) and blood pressure (BP) were sampled for 9.56 min. ± 2 days after implantation of renal nerve electrodes and arterial catheters in rat (n=3) to test the premise that low frequency BP oscillations are generated by the baroreflex. The measurements were repeated after anesthetizing the animals (pentobarbital, 30 mg/kg). SNA and BP autospectra and coherence were computed. In the low frequency range the maximum coherence (0.90 ± .002, mean ± SE) and maximum SNA power occurred at the 0.04 Hz. In contrast, BP coherence computed at the frequency (0.17 Hz) for maximum BP spectral power was only 0.40 ± 0.21. After anesthesia the frequency (0.40 ± .003 Hz) at maximum coherence and coherence (0.88 ± .004) were essentialy unchanged although SNA and BP spectral power decreased by 78.5 and 70.2 percent, respectively. These data suggest that BP spectral power at a frequency of approximately 0.4 Hz may be due to a SNA-baroreflex interaction and that anesthesia does not abolish this interaction. However, anesthesia apparently profoundly reduces both the SNA and BP responses at this frequency.
(Supported by KY Affiliate, AHA and KY Tobacco Health Res. Inst.)

495.12

Currently the etiology of Chronic Fatigue Syndrome (CFS) is unknown. It is characterized by many neuropsychological, infectious, and immunological symptoms. The most debilitating feature of CFS is fatigue that is severe enough to reduce activity by greater than 50% for longer than 6 months. Since there is no diagnostic test for CFS, arguments exist as to whether it is medical or functional in etiology. To evaluate this problem, a state paced breathing protocol of 8, 12, and 18 breaths/min both in sitting and standing was used to compare vagal power in CFS patients and normals. Vagal power was derived through the application of heart rate spectral analysis on the ECG and respiration signals. Paced breathing was lower in sitting for CFS patients vs. normals. Vagal power did not fall in the standing position as commonly seen in exercise tests on a treadmill showed an increased vagal response to exercise as compared to normals. It is not clear at this point how to reconcile these differences between these two studies. Measurement of fatigue was also analyzed using biochemical parameters through video analysis to determine if there is a correlation between physiological and functional fatigue of CFS patients and normals at similar work levels.

These results preliminarily indicate that CFS patients may exhibit a consistent organic component to their illness that is, inappropriate vagal firing in different postures and during exercise. Comparisons of the extent to which this vagal firing affects exercise may aid in the diagnosis of CFS or perhaps in the classification of severity of CFS.
(Supported by NIH # AI32447 and NIDCR # H33310030)

495.13

Simultaneous recordings of cervical sympathetic, splanchic and effenter phrenic (PHR) activity were obtained in Saffan-anesthetized, paralyzed and artificially ventilated piglets (1-38 days of age) under both anesthetic (PCO2 and end-tidal CO2). Power spectra and coherence functions of SYMP discharge were obtained by a fast Fourier transform routine. PHR discharge defined the separate inspiratory (I) and expiratory (E) epochs used for gating spectral estimates. Peaks were revealed in four frequency ranges (3-7 Hz, 10-15 Hz, 18-24 Hz, 30-38 Hz). Coherence varied from inspiratory to expiratory values (<0.1) in swine. The linear component of the age-coherence relationship for the 3 - 7 and 18 - 24 Hz peaks was significant for both I and E epochs (p <.05). The quadratic component was not significant. For the 10 - 15 Hz peak, both the linear and quadratic components were significant (p <.05). Correlation of age with E to I power ratios of the SYMP was only significant in the cervical SYMP (r = .52). Results suggest that coherence is a good indicator of postnatal maturation occurring within the SYMP system(s). (Supported by NIH grants HL-20864 and HD-28931.)

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495.14
HEART RATE DYNAMICS DURING SLEEP-WAKING STATES IN NORMAL INFANTS. R. R. Harper*, W.L. Schectman and R.M. Harper. Brain Research Institute and the Dept. of Anatomy & Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024.

Extent and pattern of heart rate variation, assessed by summary procedures, undergo marked developmental changes over the early postnatal period. Summary measures of heart rate variation, however, fail to demonstrate the beat-by-beat dynamics of heart rate. We examined the development of moment-to-moment changes in cardiac interbeat intervals in normal infants over the first 6 months of life. Twelve-hour physiological recordings were obtained from 24 normal infants at 1 week and 1,2,3,4, and 6 months of age. For each recording, plots were made of each cardiac R-R interval as a function of the previous interval (Poincare plots) over 5 weeks old to 6 months of age. The distribution of points were made for both high and low heart rates showed significant age and state effects. Analysis of covariance indicated that all state and age related changes paralleled changes in basilar heart rate; only the effect of dynamic rate (heart rate during the previous beat) accounted for differences in the distribution of points in the basilar heart rate. Thus, the relationship of one cardiac R-R interval with its predecessor changes significantly with age over this period, paralleling short and long term changes in the distribution of points. (Supported by HD22695. Data acquisition and sleep state classification were performed under the direction of Drs. J. Hodgman and T. Hoppenbrouwers under NICHD contract HD32777.)

THURSDAY AM

Poincaré analysis of heart rate dynamics. However, the strong positive correlation between one interval and the next obscures the immediate relationship between a change in heart rate and the next change. The nature of this relationship was examined in 34 infants recorded at 1 week and at 12, 24, and 6 months of age. In each sleep-waking state, the number of ARRs (the difference between two successive RR intervals) larger than 4 msec was determined as a percentage of the total number of heart beats, and each pair of successive changes was categorized based on the directions of the two changes. Analysis of variance was used to identify differences in the proportion of large ARRs and their temporal patterns over ages and sleep states. During all states, the proportion of large ARRs decreased over the first month of life and increased from 1 to 3 months of age. The reduction over the first month depended on the increase in heart rate over that period, but some subsequent changes were heart rate independent. Furthermore, during the first month of life, infants showed significantly more sustained increases in heart rate than sustained decreases, while the opposite pattern was seen in infants from 3 to 6 months of age. We speculate that the profound and enduring changes in heart rate dynamics occurring between 1 and 3 months of age may reflect the emergence of cardio sympathetic reflexes.

Supported by HD27277. Data collection and rate classification performed under direction of Drs. J. Hodgman and T. Hoppenbrouwers under contract HD27277.
CALCIUM DEFICIENCY INCREASES VENOUS REACTIVITY IN SHRs BUT DOES NOT ALTER TUBULAR RESPONSES TO NERVE STIMULATION. D.C. Hatton*, Y. Qi, and D.A. McCarron. Oregon Health Sciences University, Portland, OR 97231.

SHRs fed high Ca" diets have lower BP, less βP reactivity to NE, smaller BP responses to α1-adrenergic blockade and fewer α1- receptor binding sites in whole kidneys than SHRs fed low Ca" diets. To determine the functional correlates of the difference in renal α1 binding sites, tubular and vascular responses were assessed in the current study. SHRs fed either high (2.0%) or low (0.1%) Ca" diets for 8 weeks were used. While anesthetized with inactin (1000 mg/kg) intravenous injections of norepinephrine (NE) were made at 10, 20, 40 or 80 ng/kg. In other animals, a bipolar Ag-AgCl electrode was placed around the renal nerve. The renal nerve was stimulated at a level just below that required to change blood flow. Urine was collected for 20 min before, during and after stimulation. BP was significantly higher in animals on low Ca" diets (140 ± 22 mmHg, p < .01) as was blood flow (7.9 ml/min/gk vs. 5.6 ml/min/gk). Animals on low Ca" diets had greater reductions in renal blood flow at all doses of NE (p < .005).

Nerve stimulation caused a significant antigenicity and antiinflammatory but the changes were not different between diet groups. The results suggest vascular but not tubular responses may be altered in animals on different Ca" diets.


Intracranial administration of rat junction peptide (rJp), generated from the pro-opianomelanocortin, has a pressor effect especially in spontaneously hypertensive rats (SHR). To characterize this pressor response, we investigated the effect of angiotensin or adrenergic receptor blockers in conscious SHR. The pressor response was abolished by intracranial pre-administration of 5 μg of losartan (DuP 753) (34 ± 5 vs. 4 ± 1 mmHg). Similar blocking effect was observed by [Sar¹, Ile²] angiotensin II. The concentration of angiotensin II in CSF was increased 2.0- and 4.4-fold by 10 and 30 nmol of rJp, respectively. Pre-administration of yohimbine or propranolol did not prevent the pressor response to rJp. These results suggest the brain renin-angiotensin system may participate in the pressor response to rJp.

POTENTIAL ADVERSE EFFECTS OF LOW DIETARY COPPER AND RANDOM LIGHT/DARK CYCLES ON BLOOD PRESSURE IN NORMOTENSIVE RATS. E.S. Halas* and L.M. Klevay. Department of Physiology, University of North Dakota and USDARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202.

Humans who work a night shift have a significantly higher rate of heart disease than people who work a day shift. A disruption in this circadian rhythm may be a major cause of the increased heart disease among shift workers. However, other factors such as disrupted family life, an inadequate diet, lack of recreational opportunities, and working social shifts may contribute to the elevated heart disease. Rats were subjected to random light/dark cycles (Halas and Klevay, Soc. Neurosci. Abstract 17. 1000, 1991). The 8-hour light cycle was initiated at one of three starting periods: 12:00 a.m., 8:00 a.m. or 4:00 p.m. A given cycle would last from 2 to 4 days and then it would be changed. Half of the rats were exposed to the random light/dark cycle while the other half received the standard 12-hour light/dark cycle. Half of the animals in each group were fed a purified diet (Klevay, Am. J. Clin. Nutr. 26: 1060, 1973) with 2.00 ppm Cu diet while the others were fed the diet with 5.00 ppm Cu. There were 15 normotensive, Sprague-Dawley male rats in each of the four groups. The experiment lasted four to six weeks. Significant elevation of blood pressure occurred in the 2.00 ppm Cu groups (p < .001) whereas the random light/dark cycle groups did not exhibit any increase in blood pressure. These results are in contrast with a prior experiment (loc. cit.) which found that both the 2.00 ppm Cu diet and random light/dark cycle significantly increased plasma cholesterol. These results suggest that the environmental factors that control blood pressure and cholesterol may be different.


Recent work from our lab has provided further evidence for a role of the nigrostriatal dopamine (DA) system in the development of hypertension in the spontaneously hypertensive rat (SHR). It was shown that the release of DA in the caudate nucleus of SHR is lower than that of normotensive Wistar-Kyoto rats (WKY). In addition, in the caudate nucleus of SHR a supersensitivity of DA D1 autoreceptors was found. To further characterize the nigrostriatal DA system of SHR and WKY novel environment-induced grooming behaviour, regulated extensively by central DA systems, was studied. Moreover, receptor binding studies were performed to establish affinity and concentrations of DA receptors in both strains. Novelty-induced grooming behaviour scores were lower in SHR than in WKY. A dose-dependent suppression of grooming behaviour was induced by the DA D1 antagonist SCH 23390 and by the DA D1 agonist quinpirole. The SCH 23390-induced suppression was less, whereas by quinpirole was more pronounced in SHR than in WKY. No differences between SHR and WKY were found in binding characteristics of DA receptors. The results suggest that differences between SHR and WKY as found in the grooming paradigm are not related to changes in binding characteristics of DA receptors. However, the stronger quinpirole-induced inhibition of grooming behavior was previously reported to be a hypersensitivity of presynaptic DA D2 receptors in the caudate nucleus of SHR. This work is supported by a Vidi grant from the Netherlands Foundation for Scientific Research (NWO).
496.11
EFFECTS OF CHEMORECEPTOR STIMULATION ON REGIONAL HEMODYNAMICS IN RATS. A.M. Hoque, R.A. Shaffer and S.J. Lewis*, Dept. of Pharmacology and Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Chemoreceptor (CR) reflexes are critical in the regulation of cardiovascular function. In this study the effects of CR stimulation by sodium cyanide (NaCN 50 - 200 μg/kg, iv.) on regional hemodynamics were examined before and after bilateral transection of the carotid sinus nerves (CSNX) in urethane-anesthetized rats. The injection of NaCN produced a fall in arterial pressure (AP), an increase in cardiac output (CO) and a reduction in total peripheral resistance (TPR). NaCN also increased hindquarter (HQR), renal (RF) and mesenteric (MF) blood flows and consequently reduced the resistances in these beds. Bilateral CSNX produced marked increases in TPR, hindquarter (HQR), renal (RR) and mesenteric (MR) resistances, a decrease in CO and no change in AP. Bilateral CSNX did not modify the hemodynamic effects of NaCN. These results suggest that stimulation of chemoreceptors produces marked cardiovascular changes and that the CSNs also provide tonic regulation of regional hemodynamics. The present study also suggests that iv. NaCN stimulates chemoreceptors other than those in the CSN. (Supported by HL14388 and HL44546)

496.13
INFLUENCE OF ESTRUS CYCLE ON CONTRACTILE RESPONSES TO SENSORY AND ADRENERGIC NERVE STIMULATION IN FEMALE RAT BLOOD VESSELS. Z. Li and S.P. Buckelew*, Dept. of Pharmacology, University of California, Irvine, CA 92717

To study whether the estrous cycle has a significant effect on vascular reactivity to nerve stimulation, 3 month old F-344 rats in oestrus and proestrus stages (characterized by vaginal smear) were used. In the isolated Krebs perfused mesenteric vascular bed with guanethidine present to block adrenergic nerves and metabolites to maintain smooth muscle tone, sensory nerve activation caused by nicotine (Nic) exposure or transmural nerve stimulation (TNS) was not significantly different between mestrous and proestrus groups. In isolated tail artery rings segments which are rich in adrenergic but lack sensory innervation, increasing frequencies of TNS caused an increase in developed force in both mestrous and proestrus groups; no significant difference was seen. Furthermore concentration response curves to noradrenaline (NE) were superimposed. In addition, there was no significant difference in the non-specific relaxation produced by higher concentrations of nicotine. We conclude that different estrous stages do not significantly influence vascular responses to sensory or adrenergic nerve stimulation.

Supported by California Tobacco Related Disease Program

496.14

It is well established that Ach releases NO from the vascular endothelium. This study examined the possibility that the effects of Ach on regional hemodynamics in urethane-anesthetized rats involves NO. Ach (0.1 - 5 μg/kg, iv.) produced a fall in mean arterial (MAP) and pulse pressure (PP) and heart rate, an increase in cardiac output (CO) and decreases in total peripheral resistance (TPR), hindquarter (HQR) and mesenteric (MR) but no change in renal (RR) resistance. Following injection of the NO-synthesis inhibitor L-NAME (25 μmol/kg, iv.), Ach produced exaggerated falls in MAP, no bradycardia, an exaggerated increase in CO, similar changes in TPR and HQR but larger reductions in RR and MR. However, the duration of the Ach effects were markedly reduced following L-NAME. L-NAME also abolished the Ach-induced reduction in PP. These results suggest that a) NO is involved in the Ach-induced bradycardia and the maintenance but not the initiation of the hemodynamic effects of Ach, b) Ach reduces PP (probably via a decrease in vascular compliance) by a NO-dependent mechanism. (Supported by HL14388 and HL44546)

496.15

Bretylium (BRE) selectively depolarizes sympathetic nerve terminals leading to a release of neurotransmitter stores. This study examined the effects of BRE on vascular hemodynamics in conscious rats. BRE (5 mg/kg, iv) caused an increase in arterial pressure (AP), a sustained decrease in hindquarter resistance (HQR), and a gradual increase in renal (RR) and mesenteric (MR) resistances in saline-treated rats. After the adrenoceptor antagonist prazosin (100 μg/kg iv), BRE produced hypotension and exaggerated decreases in HQR, RR, and MR. The NO synthase inhibitor L-NAME (25 μmol/kg iv) abolished the BRE-induced decrease in HQR in saline-treated rats and the decreases in HQR, RR, and MR in prazosin-treated rats. This suggests that BRE causes the co-release of pre-formed stores of noradrenaline and vasodilator NOFs from sympathetic nerves. This raises the possibility that these are neurotransmitters in sympathetic vasodilator neurons and neuromodulators in sympathetic vasoconstrictor neurons. (Support by HLB 14388 & 44546)

The so-called dorsal facial area (DFA) is located at levels from 5 mm to 7 mm rostral to the obex and dorsal to the facial nucleus in the medulla in cats. Either electrical or glutamate stimulation of the DFA in the anesthetized cats induced predominantly an increase of blood flow of the ipsilateral common carotid artery, accompanied with neither change in blood flows of other vascular beds nor changes in the heart rate and blood pressure. The DFA response were mediated via the 7th and 9th cranial nerves. By measuring tissue blood flow (TB) with radiolabeled microspheres reference flow technique, we found both the intra- and extracranial TB were all increased in the DFA response. In addition, the DFA vasodilation involved atriope sensitive and insensitive mechanisms. Results of immunocytochemical studies showed that CA1, CA3, and CA2 DBH immunoreactive somas in the DFA. The substance P and serotonin immunoreactive fibers also presented in the DFA.


Microspheres reference flow techniques was used to measure regional blood flows (RBFs) of the intra- and extra-cranial tissues. Electrical or glutamate stimulation of the dorsal facial area (DFA) generally increased the intracranial RBFs of both cerebral hemispheres. Intracranial blood flow increases were preceded after administration of atropine but reduced after physostigmine. In contrast, extracranial blood flow increases responded to both drugs in the opposite direction. Thus, DFA stimulation may cause acetylcholine release to promote extracranial RBFs increase, but to restrict intracranial RBFs increase.


Questions remain as to the physiological role of RI adrenergic cells within the RVLM in the maintenance of arterial pressure (AP) at rest and during daily stresses. The instrumented dog has been our model for recording cardiovascular responses before and after incomplete lesions are made in pressure regions of RVLM by microinjection of kainic acid. Understanding the central command component of cardiovascular control during dynamic treadmill exercise (TET) is the purpose of this study. Mongrel dogs (n=15) were conditioned to run a modified Bruce protocol up to 4 mph. 16 % grade during which heart rate (HR), AP, aortic blood flow and cardiac output (CO), internal thoracic arterial flow (IT) and total peripheral resistance (TPR) were monitored. Instrumentations included solid state pressure transducers (Konigberg) and pulse-transit time flowmeter (Transonic) and 20 MHz Doppler probes. With a unilateral lesion (100 nl, 100nmol), in the transition region between the compact division of n. ambiguous and retrofacial n. the cardiovascular pattern response to exercise was unchanged. AP was significantly increased (P<0.01) and did not even attain pre-exercise resting values during the TET. TPR was significantly lower at all workloads except 3/0. The CO, peak systolic aortic flow, and IT were consistently lower than pre-exercise values but not significantly. When observing behavioral and motor control there was no discernible difference in the pre- to post-exercise treadmill performance.

Lesions causing these results were relatively small, approximately 1mm diameter, and within the rostral portions of the CI cell columns as revealed by immunocytochemical staining for PHNT. The cardiac portions of the CI are universally important in the maintenance of AP during rest and exercise (Supported by NIH grants HL-39105)

497.4 ANATOMICAL RELATIONSHIPS AMONG THREE POPULATIONS OF MEDULLARY EFFERENTS: RETICULOHYPOTHALAMIC, RETICULOVAGAL, AND RETICULOSPINAL NEURONS. G. G. Lee, H. Hardy, Departments of Physical Therapy and Anatomy, University of Mississippi Medical Center, Jackson, MS 34621.

Within the medulla are neurons that project to the hypothalamus, the vagus nerve, and the spinal cord. The primary purpose of the present study was to identify those regions which contain representative from each of these neuronal populations. (The long term hypothesis being that those areas, containing a diversity of efferents, may serve to integrate medullary control over vital functions.) For this purpose, a variety of retrogradely-transported tracers were made into either the posterolateral hypothalamus, vagus nerve or thoracic spinal cord, in a series of anesthetized rats. Subsequently, the locations of labeled medullary neurons were plotted.

The three neuronal populations, mentioned above, were observed in close apposition within the ventrolateral (CVL) and dorsomedial (CDM) aspects of the caudal medulla. In the CVL, the three populations of neurons were observed in the vicinity of the nucleus ambiguus (NA). Vagal projections originated from the dorsomedial aspect of NA, whereas spinal projections originated from the ventrolateral aspect of NA. Hypothalamic projections did not originate from the NA, but rather from an area immediately ventral to it. In the CDM, the three neuronal populations were observed in the vagal-solitary complex.

497.5 NUCLEUS TRACTUS SOLITARIUS (NTS) HYPERTENSION IN GUANETHIDINE-SYMPATHOMIMETIZED RATS. E. E. Benaroch*, J. D. Schneler, K. K. Pickard and P. A. Lowe. Neurophysiology Laboratory, Dept. of Neurology, Mayo Clinic, Rochester, MN 55905.

Guanethidine (GU) produces chronic postganglionic sympathoexcitatory (GUS) in adult rats. We sought to determine the effects of chronic sympathoexcitatory on the development of hypertension induced by kainic acid (KA) injection into the nucleus of the tractus solitarius (NTS). Guanethidine (40 mg/kg i.p.) was administered daily for five weeks. Controls received saline, and eight weeks after treatment, rats were anesthetized (urethane), cannulated and ventilated. Arterial pressure (AP) and heart rate (HR) were continuously monitored. Plasma norepinephrine (NE) and epinephrine (E) were measured by HPLC and vasopressin (AVP) by radioimmunoassay. NE and E were injected bilaterally into the NTS by Basal AP, HR, and plasma NE and E were slightly but not significantly lower than in GUSX. KA injection into the NTS produced hypertension in both controls and AP was higher in controls. (AP: 94±5 vs. 40±9 mm Hg, P<0.01). In control, but not GUSX, rats, hypertension was associated with significant elevation of NE (from 1.8±0.3 to 1.5±4 mg/ml, p<0.05) and E (from 2.5±0.3 to 7.6±1.5 mg/ml, p<0.05). Plasma AVP was similar between groups. GUSX showed AVP, but does not present, NTS hypertension. Expression of NTS hypertension may reflect denervation supersensitivity of vascular targets and sparing of preganglionic ganglion neurons, as well as prepressor effects of circulating AVP.
497.7 AREA POSTREMA STIMULATION EXCITES THEN INHIBITS MEDULLARY NEURONS IN THE BAROREFLEX PATHWAY. A.C. Bonham*, M.A. Obadai, K. Reid and J.A. Stewart. University of California, Davis, Davis, CA 95616.

Neurons in the area postrema augment baroreflex-mediated sympathoinhibition. We have shown previously that this augmentation may occur in the nucleus tractus solitarius (Soc Neurosci 10:220, 1990). The current study was aimed to determine if area postrema neurons mediate the activity of neurons in the rostral ventrolateral medulla (RVLM) in the baroreflex pathway. We performed experiments in -chloralose-anesthetized, paracervically and vagally isolated rabbits in which renal sympathetic nerve activity (RSNA), arterial pressure, ECG and tracheal pressure were recorded. Intracellular single unit activity was recorded in the RVLM and tested for discharge patterns locked to RSNA; for baroreceptor input by injecting v phenylphrine (8 - 200ug/kg) and nitroprusside (6 - 200ug/kg); and for responsiveness to single electrical pulses delivered to the area postrema (25 - 60 Hz). Six of seven cells which responded to electrical stimulation of the area postrema were inhibited by intravenous injection of atropine (4 - 1mmg/kg), and were exited by decreases in arterial pressure (5 - 10mmHg), also received a biphasic excitatory/inhibitory input from the area postrema. The early excitatory phase had a mean peak latency of 31.7 ± 0.4ms (x±SEM) (range 24.5 - 36.3ms); the subsequent inhibitory phase had a mean onset latency of 48.1 ± 17ms (x±SEM) and duration of 84.0 ± 51.4ms (range 40.0 - 160.0ms). The remaining cell was excited (peak latency of 31.7ms) but not inhibited by area postrema stimulation. These findings suggest that area postrema may modulate the activity of medullary sympathetic neurons in the baroreflex pathway. Supported by AHA 9-136.

497.8 AREA 1 AREA NEURONS WITH PROJECTION TO THE SUPRAOPTIC NUCLEUS ARE EXCITED BY ELECTRICAL STIMULATION OF THE ABDOMINAL VAGUS NERVE Z. I. Garrobo* and W.W. Blessing. Centre for Neuroscience, Flinders University, Adelaide, SA 5042, Australia.

Neurons in the A1 area of the medulla oblongata excite vasopressin-secreting cells in the hypothalamic magnocellular nucleus. Vasopressin neurons in the hypothalamus are stimulated by action potentials also modulate the activity of neurons in the rostral ventrolateral medulla (RVLM) in the baroreflex pathway. We have now tested, in urethra-anesthetized rabbits (1.5 g/kg, i.v.), whether the discharge rate of A1 area neurons, identified with extracellular tungsten electrodes and antidromic activation from the supraoptic nucleus, meeting collision criteria, is affected by electrical stimulation of the abdominal vagus (cuff electrode at the level of the diaphragm, 0.5 ms, 200 Hz, 1.3 cathodal pulses). Peristimulus time histograms were constructed using an ITC16 interface and a Macintosh IIx computer programmed with Igor. Of 58 neurons identified, 42 (72%) were excited by stimulation of the vagus. No neurons were inhibited. The latency to maximum excitation was 251 ± 11 ms (conduction velocity 0.52 m/s). Excitation of neurons was followed by inhibition lasting approximately 50 ms. Of the 30 neurons excited by the vagus, 26 were inhibited by activation of baroreceptors using intravenous phenylphrine. We have searched for a physiological stimulus which might activate these neurons. Infusion of hypertonic saline into the portal vein did not affect the discharge of the A1 cells. Nor did distension of the stomach with a balloon. Nor did stimulation of the central end of all nerves entering the lumen of the kidney. These results provide evidence, that nearly all neurons in the A1 area with projections to the supraoptic nucleus are excited by electrical stimulation of the abdominal vagus. The relevant physiological stimuli activating the cells via vagal afferent fibers are not yet identified.

497.9 CARDIOVASCULAR EFFECTS OF OXYTOCIN INJECTIONS INTO AREAS OF THE MEDULLA OBLONGATA RECEIVING OXYTOCINERGIC INNervation. R. E. Gomez, M. A. Cannata, M. Anwar* and D. Ruggiero. INSNSA, DBA, RS. As. and Div. of Neurobiol, CUNY, NY.

We examined the distribution of oxytocinergic terminals and the effect on arterial pressure (AP) of oxytocin microinjections into the nucleus tractus solitarii (NTS) and the reticular formation of the caudal RVLM and rostral (RVL) ventrolateral medulla. Immunocytochemical labeling with an antisera against -synthetic oxytocin revealed axons and punctata with oxytocin-like immunoreactivity in NTS and RVLM. In combined retrograde transport immunofluorescence in rats injected with a tracer into hypothalamic nucleus or RVLM, Gold into NTS labeled larger numbers of neurophysiological neurons in the parvicellular and paraventricular hypothalamic nucleus than RVLM deposits. Changes in AP (mmHg) after bilateral microinjections in anesthetized rats occurred within the first minute after injection: Oxytocin NTS 10 pmol Cvl 20, 0.2±0.05 0.2±0.05 0.2±0.05 0.2±0.05 0.2±0.05

A similar dose of oxytocin injected intravenously produced no change in AP. The data suggest that oxytocin may control AP via receptors in the medullary reticular formation.


Electrical stimulation (10sec, 5mA, 20-100µA) in the lateral and dorsal periaqueductal grey matter (PAG) evoked a pressor response with tachycardia and vasodilation in hindlimb muscles in urethane-anesthetized rats. Activation of perikarya in nucleus raphe magnus (NRM) and nucleus raphe obscurus (NRO), by microinjection of 5-10nmol 5-bromo-homocysteinyl (BLH) attenuated all components of the PAG-evoked cardiovascular defence response whereas individual components of the LAAM-evoked response were modified selectively. Stimulation in NRM reduced the LAAM-evoked vasodilation by 64%, converted the tachycardia to a bradycardia and had no significant effect on the pressor response. Activation of cells in NRO also produced a reduction (38%) in the LAAM-evoked pressor response but potentiated the tachycardia and increased resting MAP by 150% and 35% respectively. The results suggest: (1) that the medullary raphe may differentially modulate activity in the descending pathways from the hypothalamic and midbrain defence areas and 2) that the central differential between NRM and NRO with respect to control of the LAAM-evoked response.

497.12 TRACING OF SINGLE FIBERS DEMONSTRATES THAT MIDDIBRAIN PERIQUADUETAL GRAY NEURONS HAVE COLLABORATED PROJECTIONS TO SOMATIC AND CARDIOVASCULAR RELATED REGIONS IN THE MEDULLA. P. Carrive, R. Bandier and M. Christie* Departments of Anatomy and Pharmacology, University of Sydney, NSW, 2006, Australia.

The midbrain periaqueductal gray matter (PAG) plays a crucial role in the integration of somatic and autonomic components characteristic of different patterns of defensive responses. These components are mediated by descending projections to autonomic and somatic regions of the lower brainstem, but the extent of collateralization within these projections is not known. We report here the existence of collateralized projections to distinct somatic and autonomic regions of the medulla and cervical cord. Biocytin was used as an anterograde tracer. It was applied iontophoretically at sites located in the intermediate third of the lateral PAG of 3 rats and 2 cats. Ten fibers labelled with biocytin were followed from section to section and reconstructed along their entire medullary course. The results show that 6 of 8 fibers terminating in the vasopressor region of the rostral ventrolateral medulla also have collaterals terminating (i) in the dorsomedial part of the facial nucleus (control of ear musculature; 3 fibers), (ii) in the nucleus retroambiguus (control of expiratory musculature; 3 fibers), and (iii) in the ventral horn of the upper cervical spinal cord (control of neck muscles; 2 fibers). Each region mediates a characteristic component of the defensive reaction evoked from the intermediate third of the lateral PAG. Such collateralized projections may well play a significant role in the integration of the autonomic and somatic functions characteristic of this region of the PAG.

497.13 LONGITUDINAL COLUMN OF CARDIOVASCULAR SYMPATHETIC NUCLEI IN CAT MEDULLA EXTEND MEDULLALLY INTO LATERAL SENSITORY FIELD. C.W. Hensens*, B. E. Robertson, and C.J. Fontana. Lab. of Neurosurgery, Tulane University School of Medicine, New Orleans, LA 70112.

We have previously shown (Neurosci. Abstr. 17: 994, 1991) that cardiovascular sympathetic nuclei in cat medulla are distributed in a longitudinal column parallel and lateral to the longitudinal array of cells comprising the ambiguous nucleus. The column extends 4.5-5.0 mm from its posterior end in the caudal ventrolateral medulla (CVLM) to its anterior end above the rostral ventrolateral medulla (RVLM). In contrast, Gebber and Herrman have reported in cat a similar parasagittal distribution of cells displaying cardiovascular sympathetic activity, but lying entirely medial to the ambiguous nucleus in the lateral sagittal column (J. Neurophysiol. 54: 1498, 1985). We now report that a survey of the medullary region lying medial to our lateral column, using microinjection of excitatory amino acids, verifies the existence of a continuum of cardiovascular sympathetic nuclei cells distributed from 2 to 4 mm laterally and encompassing the region of the ambiguous nucleus. Chemical inhibition of small areas of this continuum yields hypertension, tachycardia, and partial loss of baroreflex, with these effects weakening in the more medial and caudal aspects of the array.

Stimulation of the hypothalamic defense area (HDA) elicited a cardiorespiratory response that is thought to prepare the animal for “fight or flight.” This response is characterized by increases in cardiac output, blood pressure (BP), heart rate (HR), hindlimb blood flow and hyper ventilation. The cardiorespiratory response elicited by stimulation of the vigilance area (HVA) is associated with the inhibition of movement and is characterized by a BP increase, HR decrease and brief inspiratory apnea or a shallow tachypnea. The present study was conducted to determine if HDA and HVA stimuli give rise to different responses with NTS in rabbits.

Adult New Zealand albino rabbits were anesthetized with isoflurane. The HDA and HVA stimulation was delivered to the posterior dorsomedial hypothalamus via a bipolar stainless steel electrode (200-300 μA, 100 Hz, 0.5 ms duration, 0.5-1.5 sec train). Extracellular unit recordings were made in NTS with stereotrode electrodes during the hypothalamic stimulation.

Most of the NTS neurons affected by electrical stimulation of the HVA showed a decrease in firing rate. Approximately half of the NTS neurons that responded to the HDA stimulation showed an increase in firing rate. Many of these NTS neurons were able to receive this information as well. A small number of the NTS neurons recorded did not respond to either the HVA or HDA stimulation. These findings suggest that both HVA and HDA make functional connections with the NTS. (Supported by NIH HL 36588 and HL 07426).


Experiments using Cholera Toxin-Horseradish Peroxidase (CT-HP) as a tracer have delineated the region of baroreceptor and cardiac afferent input as well as the distribution of vagal preganglionic nerve fibers (Eccles and Ito, 1969). However, the location, extent and number of interneuronal groups subserving the medullary component of the baroreceptor vagal reflex is unknown. In order to address these questions we have used the intracisternal Bartha strain of the pseudorabies virus (PRV) as a retrograde trans-neuronal (putatively trans-synaptic) tracer. PRV was injected into two distinct cardiac sites shown by injections of CT-HP to receive vagal preganglionic innervation: between the mainstem bronchus and the aorta and at the crossing of the jugular vein, left ventricle and pulmonary artery. The earliest labelling of neurons in the medulla was present at 48 hours and was confined to members of the nodal and cranial cardiac sympathetic (NA) vagal cardiac efferent populations, as defined by location and morphology seen earlier in the CT-HP studies. With longer survival times the picture became much more complicated, but we will focus here on just a few relevant cell groups. At 68-72 hours survival time numerous non-vagal motor neurons were labelled with PRV in the vicinity of the cranial NA group, basically in the area of the cranial ventro-lateral medulla or A1 cholinergic population. Labelled neurons were scattered along an arc through the dorsal medullary reticulotegmental field and a few scattered neurons were labelled in the nucleus tractus Solitarii (NTS), particularly in its ventral subdivisions. At 94 hours this later labeling became more dense, and in a dorsal medullary group appeared in the dorsal NTS within the regions receiving baroreceptor and cardiac afferents. We interpret these results to suggest that the simplest baroreceptor vagal reflex involves at least two interneurons.


We previously characterized two groups of rhythmically beating cells at 5Hz (autonomic & sympathetically driven) in the nNTS in vivo (Patton et al. J Neurophysiol. 60: 854-858, 1987). The present study sought to determine whether such neurons were found in vitro and, if so, to physiologically characterize cells based on the varicosal origin of synaptic input(s). Extracellular recordings were made from 28 rhythmically discharging single units in the nNTS in anesthetized rats (chloral hydrate: 375 mg/kg & pentobarbital: 75 mg/kg, i.p.). All cells fired a single spike per breath (4-6 Hz) and were divided into two groups based on their firing relationship with the wave of the ECG. One group of neurons (n>10) discharged with a relatively constant phase angle to the R wave whereas the second group was tightly coupled to the cardiac cycle but fired at a similar frequency as heart rate. Both groups of cells showed a peak in the R wave triggered histogram (mean latency: 16ms). Of 18 cells tested, 16 received an excitatory synaptic input following electrical stimulation of the ipsilateral vagus nerve (mean latency 20 ms) and 3 neurons were also excited by aortic nerve stimulation (latency range: 15-18ms). Brief periods of cardiac arrest, produced by electrically stimulating the peripheral end of the cut vagus nerve, decreased the firing frequency and, following recovery of heart rate, disrupted the phase relationship with the wave of the ECG. Verrassend (5-20 μg; i.v. bolus) strongly excited all 4 rhythmically firing cells tested. These data indicate the presence of neurons beating rhythmically at the cardiac rate in the nNTS that receive cardiac afferent synaptic inputs.


There is a paucity of information concerning the response patterns of second order NTS neurons to graded arterial pressure changes. Thus, the present study examined the firing response characteristics of presumptive second order baroreceptive neurons during increases in arterial pressure following i.v. administration of phenylephrine (PE; 5-20 μg). Anesthesia was induced in rats using halothane followed by an intraperitoneal injection of Saffan (12 mg/kg i.v.). Extracellular recordings were made from 19 single NTS neurons receiving a short and invariant excitatory synaptic input following ipsilateral aortic nerve stimulation (range of latency to spike: 1.8-4.0 ms). The responses of 19 cells tested 10 were silent at baseline mean arterial pressure of 90-120 mmHg. The response of these neurons to PE injection was characterized by either a single action potential or a transient burst of activity (1-2 spikes) at a specific time point following the rising phase of the blood pressure response. As blood pressure continued to rise past this point firing ceased until the same blood pressure level was reached during the falling phase, at which time a similar pattern of discharge occurred. Recording 9 cells showed ongoing activity (mean pressure range 90-120 mmHg) which increased and decreased proportionally during the rising and falling phases of the blood pressure response to PE injection respectively. These results show a heterogeneous population of putative second order baroreceptive NTS neurons. It suggests that while some NTS neurons reflect mean arterial pressure in their individual firing frequencies, other neuron types may contribute to population encoding of mean arterial pressure and changes in blood pressure.

497.17 IONIC CURRENT MODEL OF DELAYED EXCITATION IN NEURONS OF RAT MEDIAL NUCLEUS TRACTUS SOLITARIUS (mNTS). M.G. Andreasson, G. Khushalani, J.H. Schiff, M. Yang, D.L. Kneuss, and J.W. Clark. Oregon Health Sciences Univ., Portland, OR 97201, Rice Univ. and Baylor College of Medicine, Houston, TX 77025.

Anatomical studies suggest that aortic arch baroreceptors synapse within a limited area of mNTS. Our studies focused on intracellular recordings from medullary slices including this area and on patch clamp work with axons dispersed from mNTS. In slices, depolarization current injection resulted in a rapid increase in spike rate followed by spike frequency adaptation (SFA). Pre-hyperpolarization induced a prolonged delay (DE) before resuming spiking and eliminated SFA in all neurons. In isolated mNTS neurons, two hyperpolarization-activated, transient-outward potassium currents were found with time constants matching the DE time course. We developed a comprehensive ionic current model with thirteen coupled, first-order, non-linear differential equations (Hodgkin-Huxley) to represent 8 ionic conductances, two ATPase pumps (Na-K and Ca), a Na/Ca exchanger, and calcium balance within the neurons. With a single fixed set of fit parameters, the model effectively predicted neuronal responses to a wide range of multistep current injection protocols over tens of seconds. The voltage dependence of the ionic channels was accurately reproduced. The model demonstrates the dynamic interaction of a family of voltage dependent conductances, emphasizes the pivotal role of ionic conductance responses and suggests a valuable tool for integrating information from isolated cells with more intact preparations.

497.18 THE INTERRELATIONSHIP OF RENIN-ANGIOTENSIN SYSTEM AND ADENOSINE IN THE BRAINSTEM NUCLEI OF RATS. H. Lin, C.K. Tsai, W.C. Lin and S.H. Shih. Dep. of Pharmacology, Physiology, Psychology and Zoology, National Defense Medical Center and National Taiwan University, Taipei, Taiwan, R.O.C.

The purpose of this study was to investigate the possible interaction of adenosine and renin-angiotensin system in the brainstem nuclei of the rat. Male Sprague-Dawley rats were anaesthetized with urethane. Adenosine, angiotensin (ANG) II, ANG III, and their antagonist 1,3-dipropyl-8-p-sulphophenylxanthine (DPPX) and Sar1,Ile2 ANG III were microinjected into the nucleus tractus solitarii (NTS) and their postrema (P) or rats. Our results demonstrated that microinjection of DPPX significantly attenuated the depressor and bradycardia effect at low dose (9.6 pmol) of ANG II and ANG III. Whereas, the same dose of DPPX significantly potentiated the pressor effect at high dose (480 pmol) of ANG II and ANG III in the NTS and AP. The depressor and bradycardia induced by high dose ANG II and ANG III were slightly inhibited by DPPX. On the other hand, when ANG antagonist were microinjected into the NTS, they failed to change blood pressure. The injection of adenosine, we found that Sar1,Ile2 ANG III can affect the depressor and bradycardia effects of adenosine in the AP, not in the NTS. Modulatory mechanisms remain to be clarified.

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497.10


A critical role for the dorsal and ventral medulla oblectangia in cardiovascular regulation is well established. Further, angiotensin (Ang) peptides and receptors have been demonstrated in these cardiovascular regions. We showed an increase in expression of angiotensinogen (Aogen) mRNA in aortic-lesioned hypertensive rats compared to sham-operated controls (Nishimura et al., Hypertension, 1992). To further define more discrete changes in Ang mRNA expression in these medullary areas, we show that in situ hybridization histochemistry to both localizing and quantize changes in Ang mRNA. Aogen-specific probe was a 30-base long single-strand oligonucleotide with a 3'-terminal dATP covalently linked with the nucleotides radiolabeling Ang I (antisense). Non-specific (sense) probe was its inverse complement. Following autoradiography, signals were quantified by scintidensity to measure relative total density in the area postrema, nucleus tractus solitarius, dorsal motor neuron of the vagus (DMNX), hypothalamic nucleus, nucleus of tractus solitarius, and ventral medulla oblectangia. Expression of Aogen mRNA was increased by approximately 50% in the DMNX and the hypothalamic nucleus six days after acute lesion, at a time when circulating and central renin-angiotensin systems are activated and angiotensin II levels are elevated. Results of these studies confirm a role for the DMNX during the development of hypertension, and further indicate that compensatory homostatic systems are likely to assume a more prominent role in regulating expression of Aogen mRNA in the medulla oblectangia during the chronic stages of hypertension. (Supported in part by NIH HL-6835).

498.1

QUANTITATIVE STUDY OF THE PATTERN OF REINNEVATION OF VIBRISSAL FOLLICLES AFTER PARTIAL DENERVATION OF THE WHISKERPAD OF ADULT MICE. W.C. Corbey, E. Wecker, H. Van der Loos, B.M. Biedermann.* Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

Partial denervation of skin in adult animals leads to changes in the central representation of the neighboring skin areas. We have quantified the peripheral innervation of the caudomost whisker follicles of rows B, C and D at varying intervals after denervation (double ligature followed by transection of the nerve) of follicles of rows C and D at varying intervals after denervation (double ligature followed by transection of the nerve) of follicles of row C in adult mice. Controls show a caudo-cranial gradient (c->r) of innervation density (figure). Numbers are mediated on counts in 3 mice per group. At five days post lesion (p.l.) Wallerian degeneration controls 15 d.p.l. 100 d.p.l. is complete: there are follicles in the follicle columns of row C. At 15 days p.l. reinnervation appears and from about 60 days p.l. onwards the number of fiber nerves reaches a plateau. The gradient is reversed in reinnervation density. There were no changes in the innervation of the neighboring, intact follicles. These observations provide evidence of a numerical regulation of the reinnervation of follicular tissues and will be used to study the mechanisms underlying adult plasticity in the somatosensory pathway. Support: Swiss NSF 31-30532.

499.3


We are studying the response of the trigeminal nuclei (TN) to injury. Previous studies have shown argyrophilia and degenerative alterations of axons and terminals within the TN following pulp lesions performed in the adult cat. The effect of similar lesions performed on deciduous teeth in kittens has not previously been reported. Pulp excision of the canals were performed on the left maxillary and mandibular deciduous cusps of 8-10 week old kittens (8 subjects) with survival periods ranging from 1 day to 3 weeks. Tissue from B (Nearfus et al. TN) was reacted with the Fink-Heimer stain and in addition laminae I and II of parai caudalis/modularly dorsal horn was examined with electron microscopy. In both regions, axons and terminals were known to terminate. Examination of the Fink-Heimer preparations showed a lack of argyrophilia. Sections examined with the EM revealed axonal and terminal alterations suggestive of degeneration and less commonly of regeneration or recovery and the latter were characterized by the presence of agranular reticulum. Most of the terminal alterations were electron lucent and were found with reduced synapses vesicles sometimes surrounding by enlarged glial profiles or terminals with anclusions including glycogen particles. The lack of argyrophilia and the presence of these results suggest a greater potential for recovery in the immature feline trigeminal system following injury. (Supported by NIDR Grants DE00219 & DE04942. L.E.W. is a research affiliate of the CDMRC).

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498.5 RESPONSES AND THALAMIC PROJECTIONS OF THERMORECEPTIVE NEURONS IN RAT SUPERFICIAL MEDULLARY DORSAL HORN (MDH), W.D. Hutchison* and J.O. Dostrovsky. Dept. Physiology, Univ. of Toronto, Toronto, Canada M5S 3G8
Little is known concerning central processing of innocuous temperature inputs in the rat. The aim of the present study was to determine the characteristics and projection targets of all thermosensitive neurons in rat MDH that respond to innocuous thermal stimuli applied to the corneal region.
Recordings were made from 58 cold cells in the superficial MDH of chloralose-anesthetized rats. Cells were identified by responses to cold probes and radiant warmth and their responses further quantified with a Peltier thermode. The receptive fields of most of the neurons were on the upper lip (54%) or tongue (28%). Most (65%) of the neurons responded only to innocuous cooling, whereas the remainder could also be activated by mechanical stimulation although these responses were small compared to those produced by a rapid thermal change. Of these innocuous-sensitive neurons, 57% responded only to slow cooling, 25% only to noxious pinch and 17% to both touch and pinch stimuli. Many (56%) of the 27 neurons tested responded also to noxious thermal stimuli. Cooling steps (5°C) produced mostly tonic responses in 40% of the 15 cells tested, mostly phasic responses in 27% and both types in the remaining 33%.
Antidromic mapping was performed for six MDH cold cells. Stimulation was delivered at 250 μm steps as the bipolar electrode driven through the cortex. Multiple tracks at mediolateral intervals of 0.5 mm were made. The neurons were antidromically activated from thalamus with currents of 9–20 μA (0.2ms, 1Hz) at latencies between 0.8–2 ms. The 80 μA antidromic-evoked response was recorded in the caudal ventralis centralis; 2 of the 6 neurons were also activated from sites that were in medial thalamus, one within Sm. (Supported by NIH DE05404)

The thalamic VPM nucleus receives inputs from all 4 trigeminal brainstem subnuclei. We (Williams et al., Neurosci. Abstr. 12, 91') have reported that principals (PV) terminals are more likely to synapse on more proximal portions of VPM dendrites than terminals from interopolaris (SpVI). To further assess the extent to which PV and SpVI inputs to thalamus are complementary or overlapping, adult rats each received HRP injections in PV and rhodamine- or fluorescein-dextran deposits in ipsilateral SpVI. Only 8 out of 9 control rats (1 PV/1 SpVI) were recorded. All PV and SpVI cells were recorded from anesthetized rats before and after infusion of 0.037 - 0.5 μl of glutamate (0.5 M) or lidocaine (2%) into most of subnuclei caudalis and interopolaris. Sensitivities of PV cells were determined with antidromic and intracellular labeling methods. Glutamate-induced changes in PV Rs were seen in 19 of 43 thalamic-projecting cells, where 13 exhibited smaller Rs or became less responsive to peripheral stimuli and 6 displayed larger rs or more spikes per whisker deflection. Lidocaine-induced changes were seen in 5 of 6 local circuit neurons, all of which became less responsive to peripheral stimuli. In 3 projection neurons with phasic responses to sustained whisker deflection, glutamate-induced tonic excitation was reversed by lidocaine. Lidocaine-induced changes in local circuit neurons were only seen in 2 of 7 cells, where 1 expressed a RF shift and the other showed a larger RF. Lidocaine infusion also affected directional sensitivity in 3 projection neurons and altered adaptation properties in 4 others. Thus, it would appear that spinal V nociceptive inputs have robust influences on the RFs of many PV cells. DE07734, DE07734, BNS9-1931.

AUDITORY SYSTEM: COCHLEA

499.1 IS AVIAN BASILAR MEMBRANE TRULY LINEAR? A.N. Tenechin and C. Meleshkam*. Gallier Center, University of Texas at Dallas, TX 75235.
The only available measures of avian basilar membrane (BM) intensity functions indicate that its behavior is linear in pigeon (Gummer et al., Hear. Res. 29(6-9), 1987), implying passive cochlear mechanics. Evidence is presented of the existence of three main types of rate-dependent level functions (pulsating, slope-saturating, and linear) at the characteristic frequency (CF) of pigeon primary auditory fibers. These functions were obtained with 2dB steps between 0 and 90 dB SPL. Saturating fibers have broad dynamic ranges (up to 40-45 dB) and sometimes were barely distinguished from slope-saturating. Unlike mammals, type of function does not depend on the spontaneous rate or threshold at CF. Correlation between CF-thresholds and BM intensity level is (r=0.06) and statistically insignificant. Clear break points of slope-saturating fibers within narrow CF region in the same animal occur over a narrow range of intensities and may be found between 35-45 dB SPL in different animals. The results, along with the earlier described phenomenon of single-tone suppression, which could occur at SPLs even slightly below the CF-threshold may be explained by the nonlinearities of BM and active mechanics in avian cochleas.

499.2 CHARACTERIZATION OF MINERALOCORTICOID (TYPE I) RECEPTORS IN THE INNER EAR BY COMPETITION STUDIES WITH SPIRONOLACTONE. D.Z. Pivovar, J. Lu, C. Itskovitz, S. Hwang, D. Eleftheriou, J.A. Dierker. Department of Otolaryngology, Laboratory of Bio-otology, Wayne State University School of Medicine, Detroit, MI 48201.
Spironolactone, a clinically important anti-mineralocorticoid, is thought to compete with aldosterone at mineralocorticoid (Type I) receptor sites in target tissues. Since Type I receptors are present in the inner ear (Pivovar et al., Soc. Neurosci. Abstr. 17: 507, 1991), it was of interest to investigate the antagonistic action of spironolactone in auditory and vestibular fractions. Minimally injected inner ear tissues of male Hartley guinea pigs were incubated with various concentrations of spironolactone (4 x 10-12 to 4 x 10-5M) in the presence of 4 x 10-7 M aldosterone. A 500-fold excess of RU 28362 (a highly specific glucocorticoid agonist) was included to minimize binding to glucocorticoid (Type II) receptors. Paired incubations were performed with RU 28362, RU 28367, and a 2,000-fold excess of unlabelled aldosterone to determine non-specific binding. Specific binding was calculated from the difference between total binding in the absence (control) and presence of RU 28362 with "C-estrone and normalized to tissue dry weight. The IC50 values for spironolactone in the lateral wall of the basilar cochlear turn and the ampulla of the semicircular canal were 4.1 x 10-7 and 4.6 x 10-7 M, respectively, while the corresponding values of Kd were 2.0 x 10-5 and 1.9 x 10-7M.
Inhibition studies performed with spironolactone at a dose response reduction of 10-aldosterone binding, implying that in inner ear tissues this compound behaves as an aldosterone antagonist. (Supported by NIH Clinical Investigator Development Award DC 00040-01 to D.Z.P., NIH Grant T32 DC 00026, and ONR Contract N00014-88-K-0067.)

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499.6 DISTRIBUTION OF TRIGEMINAL NEURONS PROJECTING TO SPECIFIC CEREBELLAR CORTEX REGIONS IN THE RAT. J.A. Arends*, T.J.H. Briegel & M.F. Jacquin, Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104; Dept. of Anatomy, Erasmus University, 3000 DR Rotterdam, The Netherlands.
Single and multiple retrograde tracing studies using WGA-HRP, Fluoro-gold, Diamidino-yellow, and FITC- and TRITC-labeled latex bead injections into different "oro-facial" areas of rat hindbrain were used to study the distribution of trigeminal neurons that project to specific or widespread cerebellar regions. Injections targeted included the vermis/paravermis (uvula and lateral lobules II-V) and the hemispheres (paramedian lobule, Crus I and II, simple lobule). In 22 rats, labeled cells were seen in trigeminal subnuclei principals, oralis and/or interopolars, as well as the trigeminal ganglion. There was a clear relationship between the dominant receptive field type within the cerebellar injection area (vibrissa vs. non-vibrissa) and the somatosensory (Crus I, uvula) responses produced by the largest numbers of labeled cells in vibrissa regions of ventral principals and interopolars, with moderate numbers in dorsal orals following uvula injections. Tracer deposits in other non-vibrissa cortical regions produced labeled cells primarily in non-vibrissa regions of dorsal principals, oralis and interopolars. Similarly, contralateral and bilateral projections were primarily confined to the cerebellar areas and originated in rostral interopolars, caudal orals and rostral principals. Labeled ganglion cells were seen in all cases except those involving the paramedian lobule. Only a small % of the trigeminal mossy fibers branched to terminate in more than one cerebellar region. NIH NS29885, DE07734, DE07662.
499.3 DEVELOPMENTAL CHANGES IN THE RESPONSE OF THE PERIPHERAL AUDITORY SYSTEM TO TWO-TONE STIMULI. J. J. Fitzgibbons, T. H煘, and E. L. Woum, Boys Town National Research Hospital and Creighton University, Omaha, NE 68131.

Auditory nerve fiber stimulation studies have been used to characterize the mature cochlear transduction process, which is nonlinear in nature. In contrast to the adult, some immature peripheral fibers exhibit facilitation, rather than suppression (Fitzgibbons et al., ARO 14:422, 1991). This study investigates several of the stimulus parameters and neural factors influencing the expression of facilitation and suppression during postnatal development. Single unit responses of peripheral auditory neurons were extracellularly recorded using techniques. Rate differences were evaluated for test tones placed both above and below characteristic frequency (CF), with poststimulus time histograms of CF responses were observed throughout the first postnatal month, although its incidence declined with age, while suppression was observed as early as 7 postnatal days. Although all test/probe tone ratios tested were capable of producing facilitation in the youngest animals, above-CF test tones produced facilitation in a higher percentage of units than below-CF test tones.

Auditory nerve fiber stimulation was directly correlated to the degree of frequency selectivity, and facilitation was less likely to be expressed in units showing narrow tuning characteristics. The ability of the system to produce action potentials appeared to be one of the factors limiting the magnitude of facilitation, while suppression seemed to be more dependent upon probe tone rate than facilitation.

(Supported by NICHD RO1 DE007017.)

499.4 ORGAN OF CORTI PROTEIN II SEQUENCE. K. E. Jensen*, P. K. Robb, I. Thalmann, and R. Thalmann, Departments of Psychiatry and Otolaryngology, Washington University, St. Louis, MO 63110.

The distinctive structure and function of the organ of Corti probably requires the expression of unique proteins. Two such proteins, designated OCPI and OCPII, have been isolated (Thalmann et al., Laryngoscope 106:99-105, 1996). OCPII was purified by separation of 2-dimensional polyacrylamide gel electrophoresis, electroblotted to PVDF membranes, and subjected to amino acid sequencing. The determination of internal sequence was performed by subjecting OCPII to limited proteolysis with chymotrypsin, papain, subtilisin, or V8 protease, separation of the peptides by 2-dimensional polyacrylamide electrophoresis and sequencing the resulting bands. The sequence of OCPII, although incomplete, does not resemble the sequence of any previously described protein. The molecular characterization of OCPII permits the elucidation of the role this protein plays in organ of Corti function.

499.5 INFORMATION RATES AND CODING EFFICIENCIES OF AUDITORY NERVE FIBERS. DA Bodnar*, KS Rieke, RR Capranica, and B Bialek, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, and NEC Research Institute, 4 Independence Way, Princeton, NJ 08540.

Changes in the amplitude of a complex sound spectrum of a complex sound produce changes in both the spike rates and the synchronization characteristics of auditory nerve fibers in the bullfrog. Because of the simultaneous variations in these two coding parameters, it is difficult to assess how complex sound stimuli are represented based on solely on spike rate and synchronization patterns. Therefore, we have applied a new decoding analysis method (Bialek et al. 1991, Science, 252:1854), to examine additional coding properties of peripheral auditory units. With this method, an estimate of the stimulus waveform can be made from the spike train response of a neuron. In addition, this method provides estimates of the information rate and coding efficiency of a fiber's spike output. Thus, although a unit may have the same average spike rate in response to different sound stimuli, the information rate and coding efficiency of its spike output may differ— in particular, "naturalistic" stimuli may be coded with higher information rates and efficiencies than white noise stimuli. In this study, we have examined how changes in the amplitude and phase spectra of an acoustic signal affect the information rate and coding efficiency of the spike outputs of auditory nerve fibers in response to complex sound stimuli. This research is supported by MHI Grant NS-09244 to BNC and NEC Research Institute.

499.6 EFFECTS OF CHANGES IN THE AMPLITUDE AND PHASE SPECTRA OF COMPLEX SOUND STIMULI ON THE PHASE SENSITIVITY OF AUDITORY NERVE FIBERS. RR Capranica*, B Bialek, and KS Rieke, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In a recent study, we have demonstrated that auditory nerve fibers in the bullfrog (Rana catesbeiana) exhibit changes in their spike output in response to frequency changes in the relative phase angle of a single excitatory harmonic component in a multi-harmonic stimulus (Bodnar and Capranica (1991), Soc. Neurosci. Abstr. 17: 1197). In the present study, we have examined the effects of changes in the relative amplitude or phase angle of components in a multi-harmonic stimulus on the phase sensitivity of peripheral auditory units. For studies of changes in relative amplitudes we have compared cases in which the stimulus components have equal amplitudes or relative amplitudes comparable to the power spectrum of the bullfrog advertisement call. For studies of changes in the phase spectrum we have compared the cases when all the harmonic components have either a relative starting phase of 0°, 180°, or random. The results of these experiments indicate that the phase sensitivity of a unit can be influenced by the relative amplitudes and phase angles of the other components in a stimulus. In addition, we have found that units which exhibit two-tone suppression are sensitive to changes in the relative phase angle of the suppressor component and this phase sensitivity is also affected by changes in the relative amplitudes and phase angles of other stimulus components.

This research is supported by MHI Grant #M-09244 to BNC.

499.7 EXPRESSION OF mRNA ENCODING FOR ALTERNATELY SPliced SEGMENTS OF FIBRONECtin IN THE DEVELOPING RAT COCHLEA. P. J. Kolodziej, R. O. Wood*, D. V. Macpherson, and W. Hoshn and V. L. Wooda, Depts. of Otolaryngology and Medicine, UCSF Medical Center and Veterans Administration Medical Center, La Jolla, CA 92039-9112.

Recently, we have demonstrated the distribution of fibronectin mRNA in the rat inner ear. From embryonic day 18 through day 1 postpartum, intense, discrete immunoreactive foci was observed in the cochlear duct, immediately beneath the inner and outer hair cells, sites of auditory nerve fiber growth and nerve cells, respectively. Fibronectin was also found to be a major structural component within the basilar membrane throughout development. In order to determine the site(s) of fibronectin synthesis and the role of functionally distinct forms of fibronectin, we are performing in situ hybridizations on rat cochlear tissue with segment-specific mRNA probes (kindly provided by Dr. R. O. Hynes, MIT), which distinguish three alternatively spliced forms of fibronectin. Data will be presented demonstrating ontogenetic changes in cochlear fibronectin mRNA expression. This research is supported by DC139, DC386 & the VA Research Service.

499.8 SIMULTANEOUS MEASUREMENTS OF TUNING IN THE ANTEROVENTRAL COCHLEAR NUCLEUS AND DISTORTION PRODUCT OTOACOUSTIC EMISSIONS: EFFECTS OF ENDOCOCHLEAR POTENTIAL VARIATION. R. Ruhmann, D. M. Millis* and W. R. Bubel, Department of Otolaryngology-Head & Neck Surgery, Univ. of Washington, Seattle, WA 98195.

The objective of this study was to precisely evaluate the relationship between tuning characteristics and the thresholds of cochlear affers and the properties of distortion product otoacoustic emissions (DPOE). Response areas of multunit clusters in the anteroverentral cochlear nucleus and DPOEs at a rate of 30-40 dB. Stimulus levels were simultaneously measured in the adult gerbil during furosemide-induced changes of the endocochlear potential. Intraperitoneal injection of 0.5 mg furosemide had no effect on the high level DPOEs (stimulus intensity 75-80 dB SPL), but the low level DPOEs (stimulus intensity 50 dB SPL) are reduced from approximately 30 to 15 dB SPL within 15 min. Energy was fully recovered after about 60 min. Characteristics of the frequency threshold curves (FTC) after furosemide injection: (1) threshold at the best frequency (BF) increased by about 30-40 dB, (2) threshold of the FTC-tail (one octave below BF) was elevated by 8-10 dB, and (3) the FTC-bandwidth 10 dB above threshold widened from 0.2-0.4 octaves to 0.4-0.8 octaves. The dynamics of FTC deterioration and recovery are the same as for the DPOE reduction and recovery. This congruence indicates that tuning characteristics of cochlear affers are dynamically regulated by the same biological mechanism that regulates otoacoustic emissions.
499.9 DISTRIBUTION OF OLIVOCOCHLEAIR NEURONS IN THE CHINCHILLA. J.M. Weekly, W.B. Warr and B.J. Morey. Boys Town National Research Hospital, Omaha, NE 68131

Although the chinchilla is a commonly used animal in auditory research, very little work has been done on the origins and distribution of its cochlear afferents. The purpose of this study was to define and quantify these distributions using retrograde fluorescent tracer techniques. Four adult chinchillas were anesthetized and injected with 10 µg of FluoroGold (FG) or Fluorogold (FG) tracer into the cochlea. After a survival time of 6-10 days, the animal was fixed by intracardiac perfusion of 4% paraformaldehyde, the brain removed and sectioned. FG-labeled neurons were seen in the n. olivary core (N-3) or in the intralaminar plane (N=1) and studied under fluorescence microscopy. We could readily classify labeled OCN into two groups: lateral (LOC) and medial (MOC) olivocochlear neurons, as is the case in most mammalian species. Labeled OCN averaged a total of 1268 (s.d.=143) and were located mainly in the neuropil of the lateral superior olivary nucleus (LSO) ipsilaterally (mean=965.76% of total, s.d.=118) and in the contralateral dorsomedial periolivary region (DPOM) (mean=231.18% of total, s.d.=48). OCN were present in fewer numbers in the ipsilateral DPOM (mean=50.39% of total, s.d.=25) and contralateral LSO (mean=221.7% of total, s.d.=14). Although the general distribution of OCN in the chinchilla is similar to that known in other rodents, the restriction of MOC neurons to the DPOM is unique among rodent species so far studied. [Supported in part by NIH-NINDS-P60 DC00982]

499.10 EXPRESSION OF NMDA-RECEPTOR mRNA IN THE RAT AUDITORY SYSTEM. H. Kiyasu
day, R. Altun, S. Satoiya and R.A. Siegelj. Kresge Hearing Research Institute, Dept. Neurosciences, Univ. of Michigan, Ann Arbor MI 48109 and Dept. of Anatomy, University of Osaka, Osaka Japan.

There is now considerable evidence suggesting that glutamate or a related excitatory amino acid is a putative neurotransmitter that acts on an excitatory amino acid receptor. The NMDA receptor has been known to play a key role in plasticity in the CNS synapse, although its role in the cochlea and auditory brainstem has not been well established. Recently two sequences for the NMDA receptor have been reported (Mortoyd et al., 1991; Kuma et al., 1991), and the expression properties. Expression of NMDA receptor mRNA in the rat auditory system was examined using in situ hybridization histochemical techniques. For in situ hybridization studies one oligo-probe was made of antisense cDNA (2252/2269), of Mortoyd's (NMDAR-1) and another was made of the antisense cDNA (1107-1151) of Kuma's sequence (NMDAR-2). These probes were end labeled with 

499.11 HSP 72 INDUCTION WITH ACOUSTIC OVERSTIMULATION IN THE RAT COCHLEA AND AUDITORY BRAINSTEM. H.J. Lim, O.H. Jenkins, J.M. Miller*, M. Myser and R.A. Siegelj. Kresge Hearing Research Institute, Department of Otolaryngology, University of Michigan, Ann Arbor, MI 48109.

Heat shock proteins (HSPs) are induced after the exposure of cells to various metabolic and environmental stresses. In the auditory system, induction of the 72 kDa HSP has been shown with heat in the rod (Kim et al., 1999) and guttata pig (Thompson et al., 1992) cochlea as well as with hyperpnea in the rat cochlea (Miyers et al., 1992). The purpose of this study was to determine if high intensity acoustic stimulation would induce HSP 72 in the rat cochlea and auditory brainstem. Sprague-Dawley rats were exposed to 110 dB SPL, broad band noise for 1.5 hours and sacrificed 4, 6 and 8 hours after stimulation. Control animals did not receive any stimulation.

Immunohistochemical and biochemical analysis were performed using monoclonal antibodies against HSP 72 (Amersham, StressGen). Tissue for western blot analysis came from unfixed dissected cochlea, cochlear nucleus and inferior colliculus. Immunoreactivity was observed on vibratome sections of the rat brainstem, cristiform sections of cochlea and cochlea with the bony shell removed, the latter yielding surface preparations.

Western blots showed an intense 72 kDa band in the noise exposed animals compared to a very light band in control animals. Immunohistochemical results in the cochlea revealed the induction of HSP 72 immunoreactive (IR) staining of outer cells. The maximal IR staining was observed 6 hours after noise exposure. Only a few IR stained inner hair cells were seen and spiral ganglion cells were not stained. In the brainstem, noise induced HSP 72 IR labeling of neurons was observed in cochlear nucleus, medial nucleus of the trapezoid body, lateral superior olive, but not in inferior colliculus.

These results indicate that acoustic overstimulation can induce the expression of HSP 72 in the rat cochlea and auditory brainstem nuclei. HSP 72 immunohistochemistry may serve as a marker for cellular stress and potential damage. Further studies may also help to determine a protective function for HSP 72 in the auditory system. [Supported by NIDCD grants DC00724 and the Deafness Research Foundation]


Contralateral sound suppression (CSS) of distortion product otoacoustic emissions (DPOAEs) involves the activation of olivocochlear efferent fibers within the superior olivary complex (SO) (Puel & Brieskorn, Audiol Neurootol 6, 176:1713, 1991). One neurotransmitter of these fibers is acetylcholine (ACh). We studied the effects of ACh antagonists on CSS of DPOAEs to determine if ACh is involved and the receptor type. Urethane anesthetized guinea pigs with sectioned middle ear muscles were used. Perilymph spaces of cochlea were perfused with artificial perilymph (AP) and drugs at 2.5 µl/min for 10 min. After each period of perfusion the 2f1-f2 DPOAE at 5 kHz (t1=-6.25 kHz; t2=+7.5 kHz; dB SPL) was measured before, during and after a contralateral wideband noise (70 dB SPL). CSS of DPOAEs was 1.59 ° 0.15 dB. AP did not alter CSS of DPOAEs. Strychnine (10 µM), curare (10 µM) and atropine (10 µM) reversibly blocked CSS of DPOAEs. Results support the involvement of both nicotinic and muscarinic receptors in CSS of DPOAEs. (Supported by NIH grant DC-00722).

499.13 MIDDLE EAR TRANSFER FUNCTION AND DIRECTIONAL CHARACTERISTIC IN CLAWED FROG AND ZEBRA FINCH.


Acoustic properties of the middle ear were studied in the clawed frog Xenopus laevis and in the zebra finch Taeniopygia guttata. During the experiments the frog was positioned dorsal side up with the water surface on a small table in a circular 4m pond. A probe microphone was inserted into each middle ear. Reference microphone was placed on both sides of the frog's head. The zebra finch was positioned inside a sound proof chamber, on a turntable, with similar positioning of middle ear and reference microphones. In both cases acoustic stimulation consisted of continuous white noise delivered at every 10° rotation, up to 360° azimuth. Sound pressures were monitored by a special signal for analysis. Transfer functions between freefield and middle ear and between the middle ears of both sides were established for the two species. These experiments were performed under experimental conditions of intact ear and various systematic manipulations of ear structure.

Morphological investigations of the internal connection between both ears consisted of three-dimensional computer reconstructions on the basis of serial sections of the heads of birds and frogs placed in caviolin between both ears. These reconstructions were made visible by filling with plastic material. While the internal pathway of the clawed frog consisted of only one direct connection with an opening to the mouth cavity, the zebra finch possesses a more diverse internal connection that has not to be considered. The thalamus of the frog is positioned in the medial corner of the roof of the mouth; the zebra finch lies just beneath this opening. Acoustic measurements revealed a contribution of the sound pressure of the finch and not the zebra finch to the sound pressure of the freefield.

These studies also correlate to the frequency range, where sound pressure levels between the two ears were large enough for directional hearing. On the basis of the morphological and experimental results a theoretical model was designed for the description of a bird's and a frog's ear.
499.13

DIFFERENT TERMINATIONS IN THE NEONATAL HAMSTER COCHLEA. N.B. Mansdorf, D.D. Simmons*, and K. Bgl., Dept. of Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1606.

In adults, lateral olivocochlear (OC) neurons terminate below inner hair cells (IHCs) while the medial OC neurons terminate on outer hair cells (OHCs). Although both lateral and medial OC neurons are presumed to project to the cochlea at birth, it is unclear which system matures first and whether OC axons and terminals first or whether they maintain independent innervation of IHCs and OHCs. Our investigations have attempted to characterize the development of OC terminations using light and electron microscopic techniques in the postnatal hamster. Bicuculline was injected into the crossed OC bundle using an in vitro brainstem preparation. At postnatal day (P) 6, 8OC fibers gave rise to at least 2 types of endings: large dendritic swellings that terminated directly on IHCs, and smaller, more numerous, smaller terminals that terminated below the IHCs. The majority of effector endings were found on the modiolus side of the IHC. No terminal endings were observed on or below the OHCs. The developmental expression of calbindin and related peptide (CGRP), a marker specific for lateral OC neurons, was also immunocytochemically characterized. Immunoreactivity could not be detected on or before P 6 but was already present at P 9 as a diffuse stain of OPC-positive terminals only under HCs. Our data are consistent with the hypothesis that the medial OC efferents terminate on IHCs before OHCs.

(Supported by the Alfred P. Sloan Research Foundation)

499.16

DEVELOPMENT OF THE TUNNEL OF Corti IN THE RAT. L.P. Rybak*, C. Whitworth, Department of Surgery, StU School of Medicine, Springfield, IL 62794-9230.

The rat is an attractive animal which has proven to be a useful model for the study of auditory development. The purpose of the present study was to study the surface development of the organ of Corti using scanning electron microscopy (SEM). Rat pups from 1-30 postnatal days of age were anesthetized with pentobarbital and the cochleas were removed and processed for SEM using a modification of the TOTO procedure (Davis and Forge, J Microsc 1987; 147:89-101). The specimens were viewed with a Hitachi S550 Scanning Electron Microscope. The tunnel of Corti was found to undergo dramatic changes between 9 and 11 days after birth. The tunnel of Corti was extremely narrow prior to 9 days of age. A base to apex gradient in development of the tunnel width was observed. At 9 and 10 days of age the tunnel width increased in the lower middle turn and the hump region. By 11 days of age the tunnel of Corti was well developed throughout the cochlea. These morphological findings correlated with the onset of CAP response which was first detected at 13 days of age. It appears that opening of the tunnel of Corti coincides with a series of developmental phenomena which most occur prior to onset of sound-evoked responses in the cochlea.

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AUDITORY SYSTEM: ANATOMY II

500.1

INPUTS TO THE SUPERIOR PARAOLIVARY NUCLEUS. N. Kuwabara*, J.M. Zook, Dept. of Biological Sciences and OUCOM, Ohio University, Athens, OH 45701.

Projections to the superior parvocellular nucleus (SPN) were studied at the single cell level in the gerbil, mouse and big brown bat, Eptesicus fuscus, using intracellular labeling in a brainstem tissue slice preparation. As an unexpected major source of SPN input was traced from principal PC (of the adjacent medial nuclear of the trapezoid body (MNTB)). This input was in the form of collateral projections off of the main PC axon as it travels to the lateral superior olive (LSO). MNTB projections are generally ramified across the entire dorsal-ventral extent of the SPN and the terminal arborizations formed bands as extensive or more extensive than those seen in the MNTB projections to the LSO. Most of the fine en passant and terminal boutons characteristic of this projection may be associated with dendrites of SPN cells, but this has not been established conclusively. The MNTB projection to SPN is topographically organized. Principal cells located in the medial MNTB projected to the medial SPN, while those located in the lateral MNTB projected to the lateral SPN.

Other sources of input to the SPN were also labeled. Trapezioid fibers, presumably from the ipsilateral cochlear nucleus, sent branches to SPN which terminated in thin sheets parallel to the MNTB projection. The extent and pattern of these projections may distinguish the SPN from the dorsomedial parvocellular nucleus (DMPO) which is recognized in the cat and mustache bat. The DMPO but not the SPN, is a major target of collateral branches of the catipocellular axon projection to the MNTB (Morse '88; Kuwabara et al., '91). (Supported by NIDCD30130 and OUCOM)

500.2


We describe a connectionist model of the barn owl’s auditory localization system. Simulated sound is fed into an inner ear model whose output is used to generate spike activity in frequency tuned units (auditory fibers). These units feed into paired models of the nucleus magnocellularis, containing units sensitive to input phase, and the nucleus angularis, containing units sensitive to input level. These two sources, the magnocellular nuclei feed into a cross-correlation model of the nucleus laminaris whose units are therefore tuned to interaural phase differences (PID). Time averaged samples of laminar activity representing a range of interaural time differences, are fed into a model of the inferior colliculus (IC) core. Parallel, outputs from the angularic nucleus feed into a subtraction model representing a range of interaural level differences (ILL). The network integrates signals from the IC core and the VLp in its model of the IC lateral shell, which in turn feeds back to the external nucleus of the IC (ICX).

Before training, VLp units were fully interconnected to lateral shell units of similar frequency tuning. Lateral shell and ICX units were fully interconnected. Only these two sets of connections were modifiable. The network was trained by backpropagation to create ICX units with discrete auditory spatial fields as well as lateral shell units with realistic tuning properties.

By manipulating learning algorithms, sites of plasticity and training procedures we can use the network to explore details of the structure and development of the owl’s localization system.

Supported by McDonnell Pew Program in Cognitive Neuroscience.

500.3


We examined the extracellular unit activity of the ventral hyperstriatum (VHC), a neostriatal area of sensory-motor interaction in the pathway for song learning and production. Neurons in the field L complex, a thalamoimportant region of cytoarchitectonically distinct auditory nuclei, project to HVC, the 'sheer' to HVC, and surround the medial neostriatum (MNC). Injections of biotinylated dextrans into these areas retrogradely label cells throughout L1 and L3 of the field L complex. Several major pathways for auditory input to HVC. Cells in L1 and L3 have morphologies similar to types 1 and 2 cells seen in Golgi preparations. Occasionally type 3 oriented cells were labeled within L2a. Injections into HVC with which did not invade the shell appear to label more cells in L1 than in L3 whereas injections into MNC ventral to HVC label in L3. Injection that included such a shell appear to label a greater number of cells in field L than that did not.

The majority of NIF cells that were labeled by injections into HVC have dendritic arbors that are within the borders of the nucleus. Some cells, however, have dendritic arbors that extend into adjacent L1 and thus may have access to auditory information.

Some HVC cells have dendrites in the shelf. These include cells that have large somata with thick, spiny dendrites and elongate dendritic arbors, which were labeled by injections into the field L complex and presumably project to area X. Small cells with thin, sparsely spined dendrites and large cells with thick, densely spined dendrites that were labeled by injections into the dorsal arch and smaller occasionally into projection to RA, also have dendrites which invade the shelf.

Most injections into HVC labeled nearly all of the cells in NIF whereas the numbers of cells labeled in the field L complex appears to be dependent on the size of the injection site. We are investigating the axonal organization of these projections. Supported by NIH grant NS26577 to DM.

500.4


Acoustically-evoked GABA-mediated inhibition is observed in neurons in the central nucleus of inferior colliculus (IC). The dorsal nucleus of the lateral lemniscus (DLL) is a GABAergic nucleus that projects to IC. The present study examined the effects of stimulation and/or blockade of the DLL on IC neuronal firing. IC recordings were made with a microelectrode (pentobarbital, 40mg/kg ip). A cannula or bipolar concentric electrode was placed into the DLL, and the effects of stimulation and/or microinjection on contralateral IC neuronal firing were evaluated. Microinjection of a local anesthetic, lidocaine (28%), or a GABA-A agonist, THIP (10-30% dll of injection) blocked acoustically-evoked binaural or intensity-induced inhibition in most ICN neurons. The spontaneous activity of ICN neurons exhibited a dramatic reversible increase following DLL blockade. Trains of electrical stimulation of DLL resulted in reduced acoustically evoked firing in most ICN neurons, which mimicked binaural or intensity-induced inhibition. The degree of firing reduction was dependent on the intrastream frequency. Effects of DLL stimulation were blocked by microinjection of THIP (10-30% dll of injection), suggesting mediation of the effects of stimulation by direct actions on DLL cell bodies. These data suggest that the contralateral GABAergic input from the DLL is inhibitory to ICN neurons. DLL-induced inhibition in ICN neurons may be mediated, in part, by the GABAergic projection from the contralateral DLL. (Support NIH NS 21821)

We tested the hypothesis that the forebrain and the optic tectum (superior colliculus) are each capable of mediating sound localization independently of the other. The barn owl, a species with highly developed sound localization abilities rivaling those of humans. The behavioral assay for sound localization was the orientation of gaze. (Lesions can cause the expression of more complex behaviors, such as moving to a source, to be lost even when accurate localization persists, as revealed by simpler behaviors.). Moreover, both the optic tectum and the forebrain have direct access to the premotor circuitry that controls gaze.

The strategy was to disrupt the functional pathway at the level of the optic tectum and interrupt the forebrain pathway at the level of the auditory talamus, a nucleus called ovoidalis in birds (medial geniculate nuclei in mammals).

The ability of 5 trained owls to orient gaze toward sound sources was assessed with one or both structures either lesioned or inactivated with NaN0 (0.12-0.25 mg in ovoidalis; 1.0 mg in tectum). Localization responses to sources located in the contralateral (affected side) hemifield were compared with those to sources in the ipsilateral hemifield. Unilateral inactivation of the tectum caused a decrease in the probability of response, a decrease in accuracy and precision and in some owls an increase in latency. Unilateral inactivation of ovoidalis alone had little effect and, if anything, caused a slight increase in accuracy. However, unilateral inactivation of both structures left the animal responsive to sound sources located contralaterally. Thus, sound localization is processed in parallel in the tectum and forebrain. The pattern of deficits is remarkably similar to the deficits in gaze responses to visual stimuli observed in primates following neocortical and/or forebrain lesions.

This work was supported by a grant from the NID (DC 00155-12).

DIAGNOSIS AND ORTHOTOPIC MAPPING OF THE AUDITORY CORTICAL FOSSA IN DEVELOPING TREE SHREWS. E. Zimmermann, H.Bring, H. Rahmann, R. Hecke, Primatenzentrum, Schmida-Petrih, Institute of Zoology, University Stuttgart-Hohenheim, 7000 Stuttgart 70, FRG.

The development of hearing and sound perception in mammals has received increasing interest due to the scarce knowledge we have about the influence of epigenetic factors on differentiation.

Our present study is mapped and compared the representation of physically defined sounds with different biological significance in the auditory system of tree shrews by means of the ZGC-method, to analyze if and at which age these sounds induce an age-related metabolic memory of the auditory cortex.

Sprague-Dawley rats of both hemispheres were used, at which age the animals were brain-stem-specific and at which age they correspond to the pattern of adults. Besides, the development of auditory threshold from birth to adulthood, where the auditory tuning curve of the somatosensory and the auditory system was determined by psychophysical methods.

Tree shrews are born deaf, their hearing capability develops postnatally and their hearing and sound communication range is quite similar to those of higher primates (Benz et al. Behav. 109, 1989; Zimmermann, Cattabriga, J. Physiol., 1992). Discrete stimulus -- specific ZGC-patterns were discerned in the core area of the auditory cortex, the central nucleus of the inferior colliculus and the dorsal and ventral part of the cochlear nucleus in adult tree shrews. They imply a tonotopic organization. First sound-induced patterns of ZGC-uptake were visualized at the time the external meatus opens and sound-induced behaviors could be evoked. However, it was not before weaning that auditory sensitivity and sound representation correspond to those of adults. These findings suggest that experience may be involved in shaping sound perception in tree shrews. Supported by the DFG (ZI 345/5-1).

LOCATION OF AUDITORY CORTEX IN THE RAT AND FERRET: A DOUBLE-LABELLING STUDY USING 18F- AND 123I-OXYLUTEIN. M.D. Roeder, D. Hoedel, Dept. of Biomedical Sciences, AB 138 and Dept of Bio-medical Physics, AB 22D, University of Aberdeen, Aberdeen, UK.

The number and location of auditory areas in the rat and ferret neocortex is uncertain. We used the deoxyglucose method to study the distribution of control and deafened animals. Commercial 14C- and 2-deoxyglucose was injected (i.p.) (555 kbq/100 g rat or 370 MBq/100 g ferret) and the animals were exposed to normal laboratory sounds for 45 min. Some were then deafened under ether (rat) or ketamine (ferret), anaesthesia by bilateral deafferentation of the tympanic membrane.

Two hours later 18F-2-deoxyglucose produced with the University of York in a crystalstat and exposed using Kodak X-Omat-L film for 8 hours (70°C) and then after 2 days for a further 16 days (3°C). Two areas of high deoxyglucose uptake were found in the rat auditory region (area 41) and three areas on the ferret ectoeyylvian gyrus. This activity was not altered by bilateral ablation but was reduced contralaterally to a unilateral ablation.

Supported by the Mufield Foundation.

SPATIO-TEMPORAL IMAGING OF AUDITORY CORTEX OF GUINEA PIG HEARING SOUND WITH VOLTAGE-SENSITIVE-DYE. K. Fukunishi, N. Mura, H. Ueno, H. Kawaguchi*. Advanced Research Laboratory, Hitachi Ltd, Hatoyaama, Saitama 350-03 JAPAN.

The neural evoked responses which are the outputs of dynamical brain system working for each specific kind of maintained stimulation are utilized for the brain systems identification. Optimal recording seems to be an important measurement method of the neural responses which could lead to identify the brain system. A 128-channel optical recording system was developed to measure the neural responses of a animal brain for hearing sounds. The voltage sensitivity of the EROF was applied for the probe. The temporal fields of anesthetized guinea pigs (male) were observed after click and tone burst stimuli respectively. A boomerang-shaped spatial pattern due to the movement of the response parts on the auditory field was observed for the click. This kind of movement pattern was invisible for the tone bursts. The direction of the movement of the response parts for the click crossed the response parts for the tone burst. The frequency selectivity of the cortical neuron was observed but depending on the latency. This characteristics was also clarified by focusing to the strong response parts for each tone burst. The cross-correlation among the responses of each observed section revealed the existence of the modular unit of the neural information processing on the cortical field for the complex sound as the click.

FUNCTIONAL REORGANIZATION IN THE INFERIOR COLICULUS FOLLOWING ACUTE COCHLEAR TRAUMA. P.J. Salvi* and S. Saunders Hearing Research Lab. SUNY at Buffalo, Buffalo, NY 14214.

The tonotopic organization and response properties of neurons in the auditory cortex can be drastically altered when a segment of the cochlea is damaged.

However, it is unclear how neurons distal to the cortex contribute to this reorganization. In order to explore this issue, we recorded from neurons in the inferior colliculus (IC) immediately after damaging a region of the cochlea with acoustic trauma. The frequency of the traumatizing tone was located above the unit's excitatory response area in order to selectively eliminate inhibitory inputs originating above CF. Units with non-monotonic rate-level functions were unaffected by this type of exposure; however, the response properties of units with non-monotonic discharge rate-level functions were dramatically altered. Non-monotonic units responded to a broader range of low-frequency stimuli and thresholds in the tail of the tuning curve sometimes improved by more than 30 dB after the exposure. Saturation discharge rates also increased significantly (100-200%). These results suggest that the traumatizing tone can selectively inactivate regions of the cochlea which drive the inhibitory inputs to these cells and significantly alter the unit's excitatory response area and level of excitability.

THE FUNCTIONAL ANATOMY OF MIDDLE LATENCY AUDITORY EVOKED POTENTIALS: THALAMOCORTICAL CONNECTIONS. S. Di and D. S. G. BARTL Department of Psychology, University of Colorado, Boulder, Colorado 80309 USA. Recent studies in our laboratory have demonstrated that by using high resolution electrophoretical potential mapping in combination with numerical methods of spatiotemporal analysis, it is possible to identify and separate putative neural generators of the middle latency auditory evoked potentials (MLAEP) computed in the region of the auditory cortex. The object of the present study was to compare the click evoked thalamic-cortical MAEP complex with potentials evoked by direct electrical stimulation of the ventral and dorsal divisions of the medial geniculate body (MGV and MGD). Epileptiform responses to click stimulated earlier findings. The responses consisted of a positive-negative biphase waveform (P1 and N1) in the region of the primary auditory cortex and a positive monophasic waveform (P1B) in the region of secondary auditory cortex. A linear combination of these patterns was sufficient to explain from 90-94% of the variance of the evoked potentials at all latencies. In the same animals, epileptiform responses to electrical stimulation of the MGV and MGD were also localized to areas 41 and 36. A linear combination of potential patterns from these separate stimulation conditions was sufficient to explain from 80-93% of the variance of the original click evoked potential complex at all latencies. These data provide further support for the hypothesis that the defined topographical thalamocortical projections to primary and secondary auditory cortex. They suggest that short latency cortical evoked potentials (10-60 msec post-stimulus) are dominated by parallel thalamocortical activation of areas 41 and 36.
501.1 SELECTIVE STAINING OF OLFACTORY AFFERENT FIBRES IN FISHES USING ANTIBODIES RAISED AGAINST APGW-NH₂ R.Y.S. Lo and R.P. Talamo*, Neurosciences Labs, Tufts Med. Sch., Boston, MA

A new whole mount immunocytochemical method (Feng et al. 1991) was used to study the olfactory receptor neurons (ORNs) on the surface of the olfactory mucosal sheet. Studies with neuron-specific tubulin antibody, β1, of 26 specimens of human olfactory mucosa taken at autopsy from patients ranging in age from 2d to 83 yrs revealed a structure not previously described, an olfactory pit organ. In 2 infants (2d and 2mo of age), the olfactory pits appeared in 2 groups of 8-12 openings (diameter ~ 50-70 μm) within the surface epithelium (oe) located on the anterior lateral and septal walls of the nasal cavity. The openings of the pits can be oriented in different directions. In mature specimens, most of the pits were distributed near the roof of the nasal cavity. However, the number, morphology and distribution of these pit organs varied among different individuals. A detailed analysis of the structure of the pit organ was carried out by rehydrating and sectioning the whole mount specimens. The pit organ is a blind pouch lined with ORNs which appears as an invagination of the epi- dermis into the connective tissue (depth of the invagination ~ 150-200 μm). In some sections, a thick axon bundle emerging from the bottom of the pouch was observed. The extension and termination of this axon bundle in the CNS has not been explored. In specimens from aged and Alzheimer's patients, degenerative changes were found both in the oe lining the surface of nasal cavity and in the pit organs. ORNs may be sparse and the oe is often replaced by respiratory epithelium or squamous epithelium. Some pit organs have been found in monkey olfactory mucosa, but have not been observed in the human specimen. The pit organ is an olfactory receptor neurons which may play a role in odorant association with the ORN, or it may contain specialized neurons that cannot yet be distinguished morphologically or immunocytochemically. NIH AG 92020 and ADRDA IIRC-89-041

501.3 OLFACTORY MUCOSA DEGENERATION FOLLOWING TREATMENT BY THE CHELATING AGENT DIETHYLDITHIOCARBAZIDE (DDTC) Kari K. Mager, Dept. of Pharmacology and Physiology, School of Medicine, So. 111. Sch. of Med., Springfield, IL 62794

DDTC is a potent chelating agent proposed as a rescue agent in chemotherapeutic treatment of some cancers. It is hypothesized to bind excess cisplatin and facilitate its removal from tissue. As part of a study of neurotoxicity of cisplatin, we examined the effects of DDTC on olfactory mucosa. To date, five adult wistar rats have received a single dose of DDTC (18 mg/Kg s.c.). Within three days, substantial sloughing of olfactory mucosa was observed with relative preservation of the respiratory mucosa. In addition, glial response in glomeruli was substantial. These observations suggest that DDTC is toxic to olfactory receptor neurons although its function is unclear. Carnosine has been suggested to play a role in membrane stabilization and free radical scavenging. Perhaps DDTC disrupts functioning of carnosine resulting in olfactory mucosal degeneration.

501.5 MICROSCALE MEASUREMENT OF ODOR PLUMES WITH ELECTROCHEMICAL MICROELECTRODES: CONSEQUENCES FOR THE CODING OF SIGNALS FOR RECEPTOR ORN S, P.A. Moore*, R.K. Zimmer-Faust, M.J. Weissburg, S. BeMent, J.M. Parish and G.A. Gerhardt, Dept. of Pharmacology and Physiology, UCB, School of Medicine, UCB, Columbia, SC; Dept. of EE, UM, Ann Arbor, MI

The dispersal of chemicals (and hence stimulus structure) is due to the fluid dynamics of the particular marine environment. To quantify the smallest scales associated with chemical patches, we measured the structure of chemical signals under turbulent flow simultaneously with a novel microelectrode-based, multistate, microelectrochemical electrode array. Simultaneous recordings showed that patch sizes may be as small as 200 microns. Plume parameters such as pulse height and pulse slope had spatial distributions that could provide directional or distance information about the odor source to an orienting animal. Such differences in chemical signal structure over small spatial scales might be important to animals that use olfactory orientation. We propose two alternate ways in which organisms might deal with these fine scale differences in odor concentration. Animals much larger than the microscale patches may have evolved elongated olfactory organs to smooth variations in sensory input, whereas smaller animals may be able to capitalize on microscale variation to extract directional information from turbulent odor plumes. This work supported by USPHS #AG00441 and AG06344 to GAG, NSF #BMS-9013187 to RKZ-F, and ADMF training fellowship #A07464 to PAM.

501.6 MOLECULAR CLONING OF PUTATIVE OLFACTORY SPECIFIC GENES FROM CATFISH E. Betts, N. Dahmen, H.L. Wang, F. Margoliash, Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, N.J 07110.

To identify genes selectively transcribed in olfactory tissue we have used multiple strategies including differential hybridization and a cDNA library prepared from catfish olfactory tissue. Most recently we have reported on the use of functional expression of transcribed RNA in Xenopus oocytes in a search for odorant receptor cDNAs (Dahmen; Wang; Neumeci, 1991). Recent advances in automated DNA sequencing coupled with computerized access to database bases facilitate the direct use of DNA sequencing for this purpose. Plasmid DNA was isolated from 182 clones selected from a subpool of a directional cDNA library prepared from catfish olfactory mRNA. The insert were sequenced from both the 5' and 3' directions using fluorescent tagged dideoxy nucleotides and an ABI automatic DNA sequencer. The obtained sequence data were analyzed and compared to Gerhart, EMBL, and SWISS PROT data bases. Thirty independent sequences were present in the pool whose abundance varied from 1-28 copies. The three most abundant sequences were apparently not novel and similar in any previously reported sequences. An additional 14 were highly homologous to cloned sequences from other species. Among these was a homologue of a 2-subunit of a G-protein and a human tag sequence. In addition, we have identified a clone coding for a putative EF-hand calcium binding domain and one with a basic-HLH (helix loop helix) domain suggesting it may be a novel gene transcription factor. Experiments are underway to determine the tissue specificity and functional significance of these various clones.
501.7 CELL DYNAMICS OF OLFACTORY BASAL CELLS. J.M.H. Heart and J.E. Schechter. Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210

In adult rats, the olfactory epithelium has the capacity to generate new sensory neurons during normal life and after experimental injury as a consequence of the division of olfactory basal cells and the differentiation of their daughters into replacement neurons. However, the basal cell population is poorly characterized with regard to their origin, fate and maintenance in the neurogenic process. As a first step, we are investigating the kinetics of the mitotic cycle of basal cells and the fraction of the basal cell population that retains the capacity to enter the mitotic cycle. The duration of the G1-phase was determined in normal adult rats (n=3) using sequential pulse-labeling with iododeoxyuridine (IdU) followed by bromodeoxyuridine (BrdU) and was found to be 5.9 hours. Using this new information, animals were labeled with a pulse injection of [3H]-thymidine and subsequently by a 12 hour infusion of BrdU that began 12 hrs after the administration of BrdU. In both normal and methyl bromide-lesioned adult animals (4 days post exposure), basal cells labeled with both thymidine and BrdU were found, indicating that some daughter cells generated by the division of olfactory basal cells can re-enter the mitotic cycle. Experiments are underway to further characterize the dynamics of the stem cell and neuronal progenitor populations. Supported by NIH R29 DC04647.

501.9 TRANSFER OF MOLECULAR INFORMATION FROM OLFACTORY RECEPTORS TO MITRAL CELLS: ANALYSIS BY A COMPUTATIONAL MODEL. David A. Buchwald1 and Gordon M. Shepherd (Section of Neurobiology and Histology and Neurosurgery Program, Yale University School of Medicine, New Haven, CT 06510)

Mirtal cells in the olfactory bulb show differential responses to related members of a homologuous series of fatty acid molecules (Mori et al., 1992). The fatty acid sensitive cells are concentrated in one region of the olfactory bulb, implying that olfactory receptor neurons projecting preferentially to this region converge upon these cells. We constructed a compartmental model of olfactory receptors and connected it to a mitral cell model with multiple synapses. The model olfactory neurons contain an odor ligand-gated conductance situated in the cilia and voltage-gated Na+ and K+ conductances in the soma. The ligand-gated conductance displays a differential response to stimuli based upon the number of C-atoms in the fatty acid side chain. Each olfactory neuron thus responds independently in a manner reflecting the affinity of its receptor for structurally related odor molecules. One hundred olfactory neurons send axons which converge on the glomerular tuff of a single mitral cell. The mitral cell has compartments for receptive inputs and outputs and for voltage-gated conductances. The temporal aspects of the resultant input to the olfactory dendrites can be precisely controlled, as can the time course of the synaptic models. We have tested two models: either the receptor neurons express one type of receptor, or the receptor neurons are divided into 3 unequal populations, each expressing receptors with different affinities for members of the fatty acid series. With the homogenous receptor neuron population, the receptor neuron responses map relatively directly onto the mitral cell responses. By contrast, the mitral cell responses to the mixed sensory neuron population show complex patterns of spike discharges to related molecules, similar to the physiological recordings. The results are a first step toward constructing more realistic models of the initial processing of molecular information in the peripheral olfactory pathway.

IBM fellowship to (DAB) and research grants from ONR and NIDCD to (GMS).


Adult domestic pigs (Sus scrofa) show sexually dimorphic behavioral responses to the steroid pheromone 5α-androst-16-en-3-one (androstenone). Androstenone is attractive to females, and facilitates expression of a standing mating posture in estrous females. Adult males show neither response to the steroid. Olfactory sensitivity to androstenone is sexually dimorphic in humans: Adult females have lower androstenone detection thresholds, and adult males are more likely to be specifically anosmic to the odor. We measured androstenone detection thresholds in adult pigs to determine whether olfactory sensitivity to androstenone is also dimorphic in this species. To measure detection thresholds, trained pigs to perform a modified go/no-go operant task. Data from 3 females and 3 males indicated that adult female pigs' detection thresholds in our apparatus are a five-fold dilution lower than adult males (1.47 X 10^{-3} M in mineral oil vs. 2.93 X 10^{-4} M). In a second experiment (N=4) that included a control odorant, gamabutalactone, and it was found that geraniol detection thresholds for males and females do not appear to differ. The finding of a sex difference in sensitivity to this specific stimulus in pigs may prove invaluable in understanding the neural bases of odor quality coding in mammals. Supported by ADAMHA I R03 MH46457-01.

501.11 NITRIC OXIDE AND OXYGEN CHEMORECEPTION OF THE CAROTID BODY. R. R. Prabhakar*, M. Hashib and H. Cao, Department of Medicine; Case Western Reserve University, Cleveland, Ohio 44106, USA.

Recent evidence suggests that Nitric Oxide (NO) can be synthesized in mammalian cells and that it acts as an intracellular messenger in a number of neuronal and non-neuronal tissues. NO is synthesized from L-arginine by the enzyme NO synthase. Carotid bodies are sensory organs that detect the changes in arterial oxygen. Hypoxia stimulates the sensory discharge of the carotid body. The purpose of the present study is to investigate whether NO synthase is present in the chemoreceptor tissue, and if so, whether alterations in endogenous NO influence carotid body sensory response to hypoxia. Experiments were performed on anaesthetized adult cats. In the first group of experiments, carotid bodies were fixed in 4% paraformaldehyde and sectioned for histological examination of NO synthase using NADPH-diaphorase method. Many nerve fibers enveloping the glomus cells were found to be positive for NO-synthase. In addition, some glomas cells also display positive reaction for NO-synthase. The effects of blockade of NO-synthase activity on chemoreceptor response to hypoxia was examined on isolated perfused, superfused carotid bodies (n=4). NO synthase inhibitor, L-NNa (100-300 μM) enhanced the hypoxic response by 54 ± 12% of controls. L-Arginine (100 μM) reversed L-NNA-induced potentiation of the hypoxic response. In fact, in 4 of the 6 experiments, L-Arginine attenuated the hypoxic excitation by 63 ± 7% of the controls. These results demonstrate that (1) NO inhibitory modulator of the chemosensory response to hypoxia. Supported by grants NHL 38096, HL 47880 and HL 42599.


Nitric oxide (NO) has recently been identified as a novel neurotransmitter involved in glomerular toxicity and long-term potentiation. Our previous studies demonstrated that a NO-producing agent, sodium nitroprusside, stimulated cGMP production in the carotid body (Histochetistry 96: 523-539, 1991). However, the role of NO in chemoreception is unknown. The present immunocytochemical study utilized an antibody to nitric oxide synthase (NOS) to determine whether an endogenous source of NO exists in the rat carotid body. Immunoreactivity was found to occur in an extensive plexus of nerve fibers innervating parenchymal cells and blood vessels. These immunoreactive axons disappeared following transection of the carotid sinus nerve (CSN), but were unchanged following removal of either the superior cervical or nodose ganglia. NO was also present in the somata of petrosal ganglion neurons whose axons could be shown to project to the carotid body by retrograde tracers. The results indicate that the carotid body is innervated by a large contingent of NOS positive nerve fibers derived from the petrosal ganglion and that NO may play a role in modulating the functional activity of the carotid body chemoreceptors. Supported by NIH grant 12366 and 07308.

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501.13  

Carotid bodies were removed from anesthetized rats or mice for mechanical dissociation and glomus cell culture. After 2-7 days, cells were superfused with oxygenated physiological saline (pH 7.43 at 30-36°C) and viewed with phase-contrast. Rat glomus cells appeared either clustered or isolated, whereas all mouse cells appeared isolated. All cells were impaled with two micropipets. (tip < 1 μm for recording Ecm, filled with 3.0 M KCl and Vcm [K+ or Ca2+]. Filled with an exchanger of ions). Clustered rat cells had a mean K+ of 73 mM (9 × 10^-6 M, Ecm - 42 mV). In isolated cells, the mean K+ was about 32 mM (9 × 10^-6 M, Ecm - 41 ± 1 mV). The mean cell volume (measured only in 16 mouse cells, was 83 μm3 (9 × 10^-9 M, Ecm - 311 mV). After the controls, 1-25 mM Na2S2O4 was applied to reduce saline PO2 to 30-50 Torr. Hypoxia depressed most clustered rat cells and all mouse glomus cells. Most isolated rat cells hyperpolarized. When cells were depolarized, a K+ decrease by 31 mV in the clusters and by 27 mV in isolated cells. During hyperpolarization, K+, increased by a mean of 6 mV. Hypoxia had biphasic effects on Ca2+. As PO2 fell, the mean [Ca2+] increased by 41 mV, followed by an increase to 254 nM Changes in Ecm were accompanied by shifts in Ecm. When cells depolarized, the mean Ecm shifted positively by 16 mV in the clusters and by 42 mV in isolated cells. When cells hyperpolarized, the mean Ecm shifted negatively by 3 mV. Ecm same less positive (189 mV) when mouse cells depolarized. Supported by NS grants 56566 and 07090.

501.15  

Recent studies in our laboratory have shown that atrial natriuretic peptide and its analog, atriopeptin III (APIII), increase cyclic GMP (cGMP) formation in chemosensory type 1 cells in the carotid body. Furthermore, APIII and cell permeant forms of cGMP inhibit carotid sinus reflexes induced by hypoxia. Earlier studies established that hypoxia depresses basal cGMP levels in the chemosensory tissue while increasing the content of cAMP; low pH, another natural stimulus for the chemosensory body, likewise elevates cAMP content. The association between cAMP and carotid body excitation was further suggested by the finding that the adenylyl cyclase activator, forskolin, prolonged the duration of excitation evoked by hypoxia. In the present study, we have examined the influence of hypoxia, low pH and cAMP on the elevated cGMP content and CSN inhibition produced by APIII.

Rabbit carotid body was superfused with tyrode physiological media equilibrated with 100% O2 (pH 7.4) contained 6.7 ± 4.3 (SEM) pmoI cGMP/g tissue. Incubation for 10 min in 100% O2 APIII (100 μM) elevated the cGMP content 4.1-fold. cGMP in media equilibrated with 100% O2 and reduced by 19% and the response to APIII was decreased by 48% (p < 0.005). Basal levels of cGMP were unaffected at 6.8 (100% O2, no APIII), the APIII response was again diminished by 44%. The combination of pH 6.8 in 10% O2 media resulted in a total 67% decrease in the effect of APIII. In contrast, forskolin (10 μM) potentiated the APIII induced generation of cGMP by 2.0-fold. Nevertheless, recordings of cGMP activity in vitro showed that forskolin (10 μM) completely reverses APIII related inhibition. Our data suggest interactive influences between cAMP and cGMP, and that chemoreceptor activity is a consequence of their combined actions. Supported by USPHS grants NS12636 and NS07938.

501.16  

Previous neurochemical and immunocytochemical studies have shown that tyrosine hydroxylase (TH) activity in the carotid body is elevated 24-48 hr after breathing hypoxic gas mixtures. However, evidence for the induction of TH mRNA in the carotid body has only recently been obtained using in situ hybridization techniques, and little is known regarding the cellular mechanisms which regulate TH expression in the chemosensory tissue. In the present study, we have examined the influence of hypoxia on TH mRNA expression by employing either TH or tyrosine hydroxylase (TH) specific antisense oligonucleotides (ODN) linked to biotin to identify the experimental conditions which are responsible for TH induction.

Carotid bodies and superior cervical ganglia (SCG) exposed for 3 hr to superfusion media equilibrated with either 10% O2 (pH 7.4) or 100% O2 (pH 7.4) were rapidly frozen on dry ice and reverse transcriptase-polymerase chain reaction (RT-PCR) in accord with the method of Singer-Sam et al. (Nuc. Acid Res. 18: 1259). ODN designed to measure the relative accumulation of specific transcripts. The size and amount of a putative 234 bp TH-DNA product was evaluated using HPLC and agarose gel electrophoresis, and the results expressed per mg protein. Hypoxia elevated the total RNA in the carotid body 2.49 ± 0.50 fold (SE SEM), while the TH mRNA increased 3.63 ± 0.84 fold. In contrast, these parameters were unchanged in SCG similarly exposed to hypoxic media. Incubation of carotid bodies in zero Ca ++ superfusates greatly attenuated the increase in TH mRNA evoked by hypoxia (1.39 ± 0.34-fold increase; p < 0.025 compared to normal Ca ++ group). Our results suggest a direct role for hypoxia in TH mRNA induction in the carotid body, and that Ca ++ is an important component of the signal transduction pathway. Supported by USPHS grants NS12636 and NS07938.

501.17  

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the synthesis of dopamine, an important neurotransmitter. We have shown that exposure to hypoxia leads to a rapid increase in the level of TH in the carotid body. In the present study, in situ hybridization with a digoxigenin radiolabeled oligonucleotide probe was employed to determine the effect of hypoxia on TH gene expression at the single cell level. Chronic hypoxia experiments involved the maintenance of rats in a hypoxic chamber at half atmospheric pressure. Tyrosine hydroxylase expression is expressed at uniformly low levels throughout the CB. After exposure to hypoxia for 6 hr and 2 days, TH mRNA levels progressively increased in type I cells near the periphery of the CB. Intensified labeled cells were scattered throughout the CB at 7 days; high levels of TH mRNA were expressed in most type I cells at 2 weeks. In studies involving acute hypoxia, rats were exposed to a gas mixture of 5% O2, 95% N2 for three 30 min periods followed by 20 min of room air. High TH mRNA levels occurred in most type I cells of the CB. These observations indicate a different mode of response in TH mRNA levels to chronic and acute hypoxia.

501.18  

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Receptors have been identified within the gas exchange regions of the lungs of various reptiles and birds which are inversely sensitive to airway CO2. These intrapulmonary CO2 receptors (IPC), send their afferent fibers via the vagus nerve. The purpose of the present study was to characterize the dynamic response characteristics of IPC in the bull snake (Pitvophis melanoleucus). To accomplish this, we utilized a binary random function known as a pseudo-random binary sequence (PRBS) as the input signal. CO2 was randomly switched between two levels during unidirectional ventilation while IPC activity arising from a single unit was recorded. IPC responses to three CO2 step responses were measured, (3-15%, 2-1%, and 0-2%). The receptor transfer functions were derived from the frequency-domain characteristics of the best-fit ARX (auto regression with exogenous input) model to the data. The results indicated the presence of significant rate-sensitivity in receptor dynamics. However, the linear ARX model accounted for only 40-60% of the actual nerve responses. Further analysis suggested that unidirectionality in rate-sensitivity and half-wave rectification are important nonlinearities that immunocytochemical studies for the ramification of the observed dynamics. (Supported in part by NIH grants HL-02536 and RR-01861).
502.1


Deactivation of the olfactory receptor cells originating in the olfactory epithelium (OE) and terminating in the glomeruli of the olfactory bulb (OB) is not well understood. Though the rules that govern the distribution of axons to their glomerular targets remain uncertain, it is evident that a significant rearrangement of OE and OB is that axons from adjacent receptor cells in the OE do not necessarily maintain a nearby topography in the OB. In this vein, Daston et al. (Brain Res. 587: 69) reported that axons maintained normal topography in the olfactory nerve (ON) until they reached the OB. To investigate further the rearrangement of the ON we followed the patterns of small populations of axons in mesaxons using electron microscopy of serially sectioned OB.

The topology of olfactory receptor cell axons originating in the olfactory epithelium (OE) and terminating in the glomeruli of the olfactory bulb (OB) is not well understood. Though the rules that govern the distribution of axons to their glomerular targets remain uncertain, it is evident that a significant rearrangement of OE and OB is that axons from adjacent receptor cells in the OE do not necessarily maintain a nearby topography in the OB. In this vein, Daston et al. (Brain Res. 587: 69) reported that axons maintained normal topography in the olfactory nerve (ON) until they reached the OB. To investigate further the rearrangement of the ON we followed the patterns of small populations of axons in mesaxons using electron microscopy of serially sectioned OB.

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502.7

Behavioral experiments suggest that tiger salamanders (Ambystoma tigrinum) discriminate between certain reagent grade odorants (butyl and propyl acetate; butyl and amyl alcohol; butyl acetate and butyl alcohol) but do not discriminate between others (butyl and amyl acetate; butyl and propyl alcohol) (Mason and Stevens, 1981, Physiol. Behav. 26:647). To determine whether these same odorants elicit similar and/or different physiological responses in the olfactory system, we have investigated their effects on salamander OB and OB by video imaging olfactory-sensitive dye signals with high spatial (up to 256 x 256 pixels) and temporal resolution (1 frame/s). OB and OB were stained with the styryl dye 1,2-DNPEPS, which produced OB signals similar to those produced by the dye RH-414. In the ventral OB, each odorant elicited optical signals with time courses and spatial distributions similar to electro-olfactograms recorded in the same animal after the optical recordings. Propyl, butyl, and amyl acetate each elicited similar patterns of widespread activity with peaks anteriorly and posteriorly, while propyl, butyl, and amyl alcohol each elicited a much smaller area of activity with a peak anteriorly. In the OB, amyl, butyl, and propyl acetate each elicited long lasting depolarization; however, the propyl acetate response tended to be smaller and at a longer latency than the responses to the other two odorants. Propyl and butyl alcohol elicited large, long-lasting hyperpolarizations in the OB, while the amyl alcohol response was small and depolarizing. Thus, the differences in OB activity patterns evoked by these odorants paralleled the behavioral responses of the animal, while the differences in OB activity were more subtle.

Supported by USPHS, Pew Freedom Trust, and the Dept. of Neurosurgery.

502.9

Responses of rat olfactory bulb mitral cells to stimulation of the olfactory nerve layer (ONL) were recorded in vitro. Olfactory bulbs were cut into 400 μm thick slices in the horizontal plane and submerged in a recording chamber. Patch clamp electrodes (1-2 μm tip diameter) were guided into the mitral cell layer under a dissecting microscope. A bipolar stimulation electrode was placed onto the ONL, rostral to the recording electrode. Extracellular records of spontaneous and ONL-evoked activity were obtained from 75 mitral cells. Most of these neurons responded to both ONL stimulation. Some responded with one, or a short burst of spikes followed by a period of inhibition. In most cells, however, these initial events were followed by a long period (1-2 sec) of excitation. Intracellular whole-cell patch recordings were used to observe the synaptic events underlying this activity. ONL stimulation caused a prolonged depolarization accompanied by action potentials. Hyperpolarization blocked all but one spike, which preceded an EPSP. Under voltage clamp, ONL stimulation caused a long duration inward current. In some cells spontaneous EPSCs were present. High magnesium solution blocked these spontaneous currents. It is concluded: 1) There is a significant tonic excitatory synaptic input to olfactory bulb mitral cells. 2) The long period of excitation following ONL stimulation is caused by an unusually long duration depolarization. 3) ONL stimulation may generate spikes in the apical dendrites that are propagated proximally with little decrement. Supported by NIDCD DC00347 and DAMD17-91-C-1017.

502.11
ORGANIZATION OF INHIBITION ON ASYMMETRIC TUFTED CELLS OF RAT Olfactory bulb. Patrick I. Ezek and John W. Scott. Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322.

Olfactory bulb tufted cells exist in several subtypes. Of those with basal dendrites, the most superficial cells extend their basal dendrites in the direction opposite that of the apical dendrites, and their basal dendrites do not mingle with those of the more superficial cells. We made intracellular recordings of 18 biocytin-labeled asymmetric, superficial tufted cells. These cells do not appear to share granule cells with mitral cells, because they respond with small IPSPs to stimuli that activate large IPSPs in all mitral cells. At the onset of basal dendrites, asymmetric tufted cells offered a chance to study the contribution of the arrangement of dendritic shape to the organization of lateral inhibition in this system. This was investigated with localized olfactory nerve layer (ONL) stimulation. Although the effectiveness of stimulation at a particular ONL site decreased with distance from the tufted cell somata, the distribution of inhibitory influence was not determined solely by the direction of the basal dendrite. This observation, coupled with the fact that many granule cells show inhibition at short latencies by ONL stimulation, suggests that granule cells inhibiting superficial tufted cells may receive excitatory inputs from tufted cell axons. Supported by grant NIDCD 00113.

502.8
OLFACTORY BULB GRANULE CELLS DECREASE IN SIZE AND INCREASE IN DENSITY WITH EARLY Olfactory experience. J. Behbehani, C.C. Woo, and M. Leon. Department of Psychology, University of California, Irvine, CA USA 92717.

Young rats learn to approach an odor that had been experienced in the presence of tactile stimulation. Subsequent presentation of the familiar odor evokes an enhanced focal uptake of 2-deoxyglucose (2-DG) in the glomerular layer of the olfactory bulb. This enhanced uptake is associated with an increase in the focal glomerular-layer cell population (Woo and Leon, 1991). Since odors evoke a columnar response in the bulb (Guthrie, Anderson, Leon and Gall, 1991), we determined whether there were morphological changes in other olfactory layers. We investigated the size and density of mitral and granule cells found within columns both associated with the 2-DG foci and closely adjacent to those foci. We compared mitral and granule cell size and density in Nissl-stained sections from PND 19 odor-familiar pups with sections from controls. We found no difference between these groups in mitral cell nuclear area, perikaryal area, or density. Odor-familiar pups, however, had an increase in the density of superficial, but not deep granule cells within the discrimination of columns. These superficial granule cells also showed a decrease in soma size. The superficial granule cells within the foci-adjacent columns remained unchanged. A change in the density and size of superficial granule cells may contribute to the modification of olfactory bulb responses to familiar odors. These data suggest that early odor experience triggers restricted but multiple processes in the developing olfactory bulb.
502.13 THE ROLE OF THE LATERAL OLFACTORY FRACT (LOT) IN A TWO-OIR DISCRIMINATION TASK. P.K. Thomas* and R.M. Slotnick. Psychology, The American University, Washington, D.C. 2006. It has been shown that odor memory in rats can be long lived as long as the animal has sufficient exposure to the odor. Little evidence however, exists as to the mechanism of odor memory. This study determined rat performance in a 2-odor discrimination task during short (30 sec.) and long (10 min.) inter-trial intervals (ITI). Each session consisted of a novel pair of odors presented over 30 trials. Trials began with the rat being placed at the start of a runway, at the end of which were two sample ports. Each day, semi-randomly delivered one of the two odors. The trial terminated when the rat made a total of three lick responses to one port or 30 sec. had elapsed. If the rat reached the end of the runway during the 30 sec. odors, it was rewarded with 0.1 ml of water, and a correct response was recorded. A criterion of ten correct out of twelve consecutive trials was used as another measure of memory. The experimental group received unilateral lesioning of the LOT along with a contralateral bullectomy, while controls received only a unilateral bullectomy. Preliminary results show LOT lesioned made more errors per session and it took longer to reach criterion. This effect was amplified in the 10 min. ITI.

502.15 DEVELOPMENT AND DECAY OF SELECTIVE LONG-TERM POTENTIATION IN THE PIRIFORM CORTEX AND OLFACTORY BULB. J.S. Stripling* and M.P. Galupo, Department of Psychology, University of Arkansas, Fayetteville, AR 72701. High-frequency stimulation of cortical association fibers in the piriform cortex (PC) produces a selective long-term potentiation (LTP) of late components in potentials evoked in the PC and olfactory bulb (OB). The present study examined in detail the time course of the development and decay of selective LTP. Male Long-Evans rats were chronically implanted with a stimulating electrode in the association fiber system of the anterior PC and recording electrodes in the OB and a more caudal PC site. LTP was induced by 8 daily treatments of high-frequency stimulation (30 trains of 10 pulses at 100 Hz). The expression of LTP was monitored with an intracellular electrode inserted before, during, and after each LTP treatment. This testing revealed both a short-term potentiation that peaked during the daily LTP stimulation and decayed within 3 min, and a long-term potentiation that decayed very slowly and was still evident 24 hr later. The short-term component reached asymptotic levels well before the last LTP treatment, while the long-term component continued to grow in magnitude for the course of the experiment. The long-term component gradually diminished across 8 day period following the last LTP treatment, but remained at a substantial degree by repeated stimulation at 0.1 Hz (latus potentiation). A single LTP treatment given on the day after the last LTP treatment completely restored LTP to its maximal level. Our results indicate that selective LTP is a very robust and long-lasting phenomenon that persists in intact form for a minimum of 8 days with little or no decay. These characteristics make selective LTP a suitable candidate for participation in long-lasting functional changes in the olfactory forebrain. Supported by NSF grant BNS 85-19700 and the Marine Wilson Hollows Fund.

502.17 CELL TYPES IN THE PRIMARY OLFACTORY CORTEX OF THE FROG. L. Scalia* and J.T. Lettvin, Dept. of Anatomy and Cell Biology, School of Medicine, University of Kentucky, Lexington, KY 40536. Rapid Golgi and modified Golgi-Kopecch preparations of adult R. pipiens reveal the presence of four distinct types of olfactory cortical neurons not previously distinguished in earlier studies on Rana pipiens. The first is a cell whose dendrites are covered densely with moderately short spines. A second type of cell has distinctly fewer and often longer spines, mixed with club-like processes. A third cell, is aspiny. Its dendrites are beaded at irregular intervals and varicoses throughout their length. These three types of neuron have multiple apical dendrites that radiate obliquely toward the pia from a periventricular site. The dendrites of the aspiny cells often show a broader, horizontal spread within the superficial cortex. After crossing the superficial neuropil, the cortical dendrites are directed to the pial surface. Some of the cells project to the olfactory bulb, some to the second order area and some to both the olfactory bulb and the second order area. The fourth cell is a small stellate neuron, which appears sexless. Its numerous primary dendrites branch repeatedly, and the branchlets are serially decorated with closely spaced small beads resembling axonal boutons. (Supported by PHS grant RO150284).

502.18 3-HYDROXY KYNUREINE RELEASE IN PREPOMPH CORTEX: A NEURAL RESPONSE TO AMINO ACID DEFICIENCY? D.W. Gislen*, B.L. Lee and M.P. Thomas, Dept. Physical Sci. and Food Intake Lab, Sch. of Vet. Med, Univ Calif Davis, Davis, CA 95616. Recognition of amino acid (AA) deficiency induced by ingestion of AA imbalanced diets (IMB) has led to the development of PC (POMPC) test, but the mechanisms underlying this recognition are not well understood. To determine what neuroactive substances were released in POM when IMB rats were fed imbalanced diets, we have used the technique of in vivo intracerebral microdialysis (Plastics One, Roanoke VA) directed toward the POM (coordinates: A: -9.4mm; L: 4.0mm and D: 5.0mm; perfusion needle extended 1mm). After prefeeding a low protein control diet for 7 to 10 days, rats were fed IMB and POMCs were perfused with artificial CSF (11 μ/ml/min) at 5 min) by dialysis. Perforations were analyzed by HPLC with electrochemical detection. Compounds were identified by peak conformation and retention time (RT); samples were spiked with external standards to verify RT. External standards included: norepinephrine (NE), dopamine, serotonin and the metabolites: DOMA, DOPAC, DOPEG, SHIAA, HVA, LPDEPA, MNPG, 3-methoxytyramine, MOPET, normetanephrine, 3-OH-kynureline (3HK), tyramine and VMA. NE was in the noise level in all chromatograms. 3HK was significantly increased in rats treated with IMB after non-digestion of IMB vs basal at 1.5 hr: 441±65 μg/ml vs control: 201±65 μg/ml (p<0.05, paired t, N=8). Previous results suggesting that NE was increased 1.5 hr after feeding IMB were based on HPLC conditions that did not allow separation of NE and 3HK. NE and 3HK were separated by 6 minutes in the present chromatograms. We conclude that 3HK may play a role in the recognition of essential AA deficiency in the rat. Parallel electrophysiological studies are in progress using the POM slice preparation. Supported by NIH: DK35747 (UDC/Davis Clinic Nutr Res Unit) and USDA: CSRS 89 37200-5440.

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502.19
THE CELLS OF ORIGIN OF THE ENDOPRIFORM-TECTAL PATHWAY IN THE RAT. W. Chint1 and N. Shihabuddin2. 1Dept. of Psychology, Univ. District of Columbia and 2Dept. of Pharmacology, Georgetown Univ. Medical Center, Washington DC 20007.

In an animal such as the rat, olfactory stimuli play an important role in behavioral activities concerned with attention and orientation. A likely structure through which these activities may be expressed is the superior colliculus (SC). This structure, in a variety of species, has been shown to play a seminal part in the mediation of attention and orientation. There is some indication (Neasey, et al., 1986) that a possible source of olfactory information, received by the SC, originates from Endopiriform nucleus (EN). Therefore, the purpose of this initial study was to identify the cells of origin of this pathway that project to the SC.

Injections of Fluoro-Gold in the SC, besides labeling know collicular affrents, also label cells in the endopiriform nucleus (EN). Our preliminary results show that this nucleus appears to be connected to the injection site in the SC. Thus, anterior collicular injections result in faint label in the anterior EN, whereas posterior SC injections result in dense label in the posterior EN.

On basis of our preliminary findings, we are in the process of verifying this topographical connection by injecting anterograde and retrograde tracers in the EN. These experiments should not only give us a map of the distribution of terminals in the SC, but will also determine if any reciprocal connections are present between the two structures.

502.21
VOMERONASAL PATHWAYS ARE SELECTIVELY ACTIVATED DURING MATING BEHAVIOR IN MALE HAMSTERS. G. Fernandez2 and M. Meredith. Program in Neuroscience, Dept. Biol. Sci., Florida State Univ., Tallahassee, FL.

Vomeronasal (VN) sensory input is important for male hamster mating behavior. The VN system projects via the accessory olfactory bulb (AOB) to the medial & posteromedial cortical nucleus of the amygdala, the bed nucleus of the stria terminalis (BNST), and also to the medial preoptic area (MPOA), central structures shown to be important for reproductive behavior. In these experiments c-fos expression was used as a marker of neural activity to identify the pathways activated by mating behavior in intact animals, and in animals that had their vomeronasal organs removed (VNX). Sexually inexperienced male hamsters from each group were paired in clean boxes with fresh bedding and each exposed to a sexually receptive female for 45 min. After an additional 45 mins they were paired with 4% formaldehyde. Control animals from each group were put into clean boxes with fresh bedding and exposed 90 mins later. Fifty uvibestone sections were processed for immunocytochemistry using a polyclonal c-fos antibody. (Cambridge research). Results show a distinct difference in fos activation in stimulated animals compared to controls. Densely stained fos nuclei were evident in the AOB, medial amygdala, MPOA & BNST of stimulated animals. Unstimulated animals did not show this activation. VNX animals exposed to females did not mate, and had a dramatically reduced number of fos positive nuclei in all these areas. All animals (stimulated & unstimulated) showed activated nuclei in the main olfactory bulb and the preventricular thalamus, suggesting that it is not only the VN pathways and their central connections that are differentially activated as a result of mating. Ongoing studies involve double labelling for fos and LHRH to explore the participation of LHRH in the facilitation of mating behavior by VN sensory input. Supported by NIH Grant DC00096.

502.22

Female hamster vaginal secretions (VHS) strongly attract male hamsters, and stimulate copulatory behavior in males. FHVS do not have this effect on females. On a day 2-4 days prior to cell stimulation, we previously reported stimulation in the bed nucleus of the stria terminalis (BNST), medial nucleus of the amygdala (M) and the medial preoptic area of male hamsters following exposure to FMHS. In this experiment, we examined what regions within the mating behavior pathway of males differed in females in response to FHVS. Males, proestrous females and diestrus females were given a cotton swab with FHVS exposed to males, and all were examined to stimulate.

Our results indicate that the BNST in both stimulated males (n=3) and proestrous females (n=3) showed similar distribution of c-fos immunoreactive cells. Medial preoptic nucleus magnocellular (MPN-Mgn) and M showed differential distribution of immunostained cells between males and proestrus females. The differences are due to the differential steroid concentrations in cell nuclei. In proestrous females cells are decreased in the median eminence of this part of the nucleus. Within MPN-Mgn of the female, stimulated cells are decreased within the ventromedial part of this nucleus. The MPN-Mgn of males has stimulated cells throughout the whole extent of the nucleus. In this experiment we have shown that Mgn-Mpn and M are differentially stimulated in males and females in response to exposure to FHVS. These results may have significant behavior difference in the response to FHVS.

Supported by NIH R29 HD28487 and Sigma Xi.

502.23
SEX DIFFERENCES IN INDUCTION OF FOS IMMUNOREACTIVITY IN THE BRAINS OF PRAIRIE VOLES EXPOSED TO BEDDING SOILED BY CONSPECIFIC MALES VS. FEMALE VOLES. T.P. Goodspeed1, M.A. Novak1, G.I. De Vries2. Program of Neuroscience and Behavior and Dept. of Psychology, Univ of Massachusetts, Amherst, MA 01003.

Prairie voles show sex-specific responses to conspecific sexual odors. Female reproduction is stimulated by male odors and inhibited by female odors. Group-housed sexually experienced males show increased mounting behavior when exposed to odors of females in estrus. Little is known about which brain areas are stimulated when a female is exposed to conspecific odors. We identified such brain areas with fos immunocytochemistry. Male and female prairie voles were exposed to male or female soiled, or clean bedding. Two hours following exposure, males were sacrificed and their brains processed for fos immunocytochemistry. Fos immunoreactive (fos-ir) cell nuclei were counted in all mid- and forebrain areas that showed fos-ir nuclei in at least some vole brains. Significantly more fos-ir cells were found in the medial amygdala, anterior and posteromedial bed nucleus of the stria terminalis, and ventral premammillary nucleus of males and females exposed to bedding soiled by either sex. In the medial amygdala and anteromedial bed nucleus, males and females showed more fos-ir cells when exposed to bedding soiled by opposite than by same sex. In the septohippocampal nucleus, males but not females showed more fos-ir cells when exposed to bedding soiled by either sex. Although fos-ir cells were found in several thalamic areas, no significant differences were found between bedding conditions. These results indicate that there are sex differences in how brain areas respond to odors of the same or opposite sex. These sex differences may reflect the different responses of males and females to odors of conspecifics.
503.1

DIFFERENTIAL EFFECTS OF RESEEN PORE TREATMENT ON ANTHOCYTE-FACED CANAL-INDUCED EXPRESSION OF ZF268 AND C-FOS mRNA IN RAT STRIATUM AND CORTEX. Mortaliski, B. and Graybiel, A.M. Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences, Cambridge, MA 02139.

In previous immunohistochemical experiments on the rat caudoputamen (C), we found that amphetamine induces Fos-like immunoreactivity (FLI) in a striatostriatal output pattern in rostral CP, whereas cocaine induces FLI

## 503.2


Cocaine, a monoamine uptake blocker with high selectivity for the dopamine transporter, acutely induces Fos-like immunoreactivity (FLI) in striatal neurons in adult and neonatal rats. In neonatal rats, FLI was selectively for striatal cells in dopamine islands/striosomes (Johnson et al., 1992). We asked whether cocaine would induce FLI in embryonic striatum when given to pregnant mice and, if so, whether the FLI would be selective for developing striosomes. We found that cocaine (50 mg/kg or 25 mg/kg, s.c.) induced intense expression of FLI in cells of protos-striosomes in E18/E19 mouse embryos exposed to the maternal circulation, and that a similar dopamine island/striosome selective induction of FLI occurred at E20/E21. FLI-positive patches were aligned with DARPP-32-positive patches. In embryos of saline-treated dams, weak FLI was also detectable in striosomes/dopamine islands. Interestingly, cocaine induced FLI in cells that were scattered through the E17/E18 embryonic caudoputamen. It has been proposed that future striosomal cells in postnatal rats identified by early projections to the substantia nigra by Fishehill and van der Kooy, 1987) first are scattered at E19/E19 and later aggregate into patches corresponding to future striosomes. We propose that future striosomal cells may already have a functional phenotype (responsiveness to maternally transmitted monoamine stimulation) before they form patches, and that this phenotype persists through the period in which striosome clusters are formed. (Supported by M. Naisser and Angus N. MacDonald 1945 Fund)

## 503.3

SELECTIVE MODULATION OF DYE-COUPLES IN RAT STRIATAL NEURONS IN VIVO BY D2 AGONIST ADMINISTRATION. S.P. Otsu*, T.W. Berryman and A.A. Grace. Dept. of Behavioral Neuroscience & Psychiatry, University of Pennsylvania, Philadelphia, PA 19104

Using in vivo intracellular recording and Lucifer yellow staining, dye coupling in the striatum was observed in 176 (84%) of the cells injected, which was twice that reported in vitro by Cepeda et al., 1999). After i.v. administration of 350 nM dopamine (D1-3 mg/kg), we observed a significant increase in the incidence of dye-coupling (20%; **p<0.1%) as well as an increase in the extent of coupling within sets of 3-7 cells often observed after a single injection. It was unclear whether the apomorphine could have induced the enhancement of dye-coupling via a D1- and/or D2-specific action on striatal cells or by a presynaptic effect on dopamine (DA) terminals. Therefore, we examined the specificity of this response using the selective D1- or D2-specific DA agonists. Administration of quipiripine (2-3 mg/kg, i.v.) was found to increase the incidence of dye-coupling to 66% (6 out of 9 injections) and also resulted in dye-coupling among multiple cells in 3 cases, i.e. 3-5 cells were labelled. In contrast, administration of SKF 38393 (10 mg/kg, i.v.) did not significantly alter the incidence of dye-coupling from the control level (14%; 7/5). This apparent D2-specific enhancement appeared to be mediated by a postsynaptic action of the drug instead of a decrease in D1 stimulation secondary to autoreceptor stimulation since a blockage of D1 receptors by administering the D1 specific antagonist SCH 23390 (10 mg/kg, i.v.) did not result in an increase in dye-coupling (0%). Therefore, systematically applied dopamine agonists appear to exert different effects on dye-coupling within the striatum when compared to the results reported in vivo, where DA depletions were reported to increase dye-coupling. It is unclear whether DA actions on other striatal afferent systems may have played a role in this response observed in vivo. (Supported by Tourette's Foundation grant, NS36289, NS19408, MH45156 & MH43221).
503.7 EFFECT OF EXCITATORY AMINO ACIDS ON DOPAMINE AND SEROTONIN RELEASE IN THE RAT STRIATUM. T. Nakazato,1* and A. Akiyama2. 1Dept. Physiol., Juntendo Univ. School of Med., Tokyo, 2Dept. Electromech., Tokyo Inst. of Tech., Yokohama, Japan.

This study was designed to investigate whether dopamine (DA) and serotonin (5-HT) are released in the rat striatum by excitatory amino acid using microcomputer-controlled in vivo voltammetry. Glutamate (Glut), NMDA, quisquilate (Quis) and kainate (KA) (300 μM) intrastriatally, extracellular releases of DA were measured every 3 min in freely-moving rats. Glu (10−4 M, 6 μl, 24 min) and NMDA (10−4 M, 6 μl, 24 min) were intrastriatally injected, DA was released soon after the start of the injection. However, 5-HT release was not found. NMDA-mediated DA release was significantly suppressed after the administration of APV (10−4 M). Quis (10−4 M) was also administered, DA release increased in almost the same extent in NMDA-pretreated rats. NMDA-pretreated Quis-mediated DA release. KA was injected, DA release was small, although in 2 of the 5 cases releases of DA and 5-HT were much increased after the injection. This suggests that 5-HT release of KA sometimes induces striatal lesion. In conclusion, DA release in the striatum is increased by drugs in the following order: NMDA = Quis > KA.

503.8 SUBSTANCE P INCREASES EXTRACELLULAR ACETYLCHOLINE IN RAT STRIATUM. J.J. Anderson*, T.M. Engber, and T.N. Chase. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Neurons projecting from striatum to substantia nigra contain large amounts of the tachykinin substance P. These substance P-containing striatostriatal neurons also possess 5-HT receptors which control the release of substance P. Recent evidence suggests that the receptors for substance P (neurokinin-1) are selectively expressed in cholinergic neurons in the striatum (Brain Research 556:165, 1991). The relationship between substance P and extracellular acetylcholine in striatum was examined using microdialysis in awake behaving rats. Rats were implanted with chronic guide cannulas and after at least a three day surgical recovery period, microdialysis probes were inserted through the guide and into striatum. The acetylcholinesterase inhibitor neostigmine (10 μM) was included in the perfusion solution to increase the recovery of acetylcholine. Following a baseline stabilization period and collection of control dialysates, 100 μM substance P was perfused through the probe. The concentrations of acetylcholine and choline in collected dialysates were analyzed by HPLC and electrochemical detection. Substance P significantly increased acetylcholine concentrations from baseline levels in 3839 dialysates. In three out of six experiments, substance P did not elevate relative to control concentrations. These results suggest that substance P stimulates release of acetylcholine in the striatum and support an anatomical evidence of substance P receptors on cholinergic neurons in the striatum.


Excitatory amino acid receptor (EAA) antagonists have been proposed as potential antiparkinsonian agents, either alone or in combination with L-Dopa. We used the 2-deoxyglucose autoradiographic technique to examine the neural substrates for the interaction between L-Dopa and antagonists of either the AMPA or NMDA type of EAA receptor. Thus, we compared the effects of the AMPA antagonist NBQX (10 mg/kg, i.v.) and the NMDA antagonist dizocilpine (MK-801; 0.1 mg/kg, i.v.) on regional cerebral metabolic responses to L-Dopa (25 mg/kg, i.v. with 12.5 mg/kg benserazide) in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway. L-Dopa increased glucose utilization in substantia nigra pars reticulata and entopeduncular nucleus and decreased metabolic rate in lateral habenula; NBQX and MK-801 by themselves did not affect glucose utilization in these regions. Pretreatment with NBQX reduced the effect of L-Dopa in substantia nigra pars reticulata but not in entopeduncular nucleus, while MK-801 attenuated the effect of L-Dopa in both of these striatal output regions. Neither NBQX nor MK-801 altered the effect of L-Dopa in lateral habenula. These findings indicate that AMPA and NMDA antagonists differentially modulate dopamine receptor-mediated striatal output. AMPA receptor blockade appears to reduce dopaminergic stimulation of the striatoginal but not the striatoentopeduncular pathway, while NMDA blockade appears to reduce dopaminergic stimulation of both of these striatal output pathways.

503.10 POTENTIATION OF SKF 38393-INDUCED ROTATIONAL BEHAVIOR BY MK-801 IS DEPENDENT UPON PREVIOUS DRUG EXPOSURE. R.C. Boldry*, T.N. Chase, and T.M. Engber. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

Directly acting dopamine agonists produce contralateral rotations in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway. Previous studies have shown that the noncompetitive NMDA antagonist dizocilpine (MK-801, 0.1 mg/kg, i.p.) can potentiate the stimulation of rotational behavior produced by the dopaminergic D1 agonist SKF 38393 (1.5 mg/kg, s.c.), and inhibit the stimulation of rotation produced by the D-2 agonist quinpirole (0.1 mg/kg, i.p.) (Morelli et al. JPET 260:140-148, 1992). We have found that MK-801 decreases the stimulation of rotation produced by quinpirole regardless of previous drug exposure and increases the rotational response to SKF 38393 when given as a single high dose of L-Dopa (50 mg/kg with 30 mg/kg benserazide, i.p.). However when MK-801 is co-administered with SKF 38393 one week after screening with a single dose of apomorphine (0.05 mg/kg, s.c.), no potentiation of the rotational response is observed. This negative finding at one week after apomorphine is independent of the dose or route of administration of MK-801. Since rats which have been treated 3 days previously with L-DOPA display significantly less rotational behavior than those treated 1 week earlier with apomorphine, we have concluded that MK-801 can reverse the suppression of rotational behavior caused by recent exposure to L-DOPA.

503.11 MK-801 DIFFERENTIALLY MODIFIES REGIONAL CEREBRAL METABOLIC RESPONSES TO D1 AND D2 DOPAMINE AGONISTS. S.M. Papa, R.C. Boldry, T. Anderson, P. Cornish, T.N. Chase, and T.M. Engber, ETB, NINDS, NIH, Bethesda, MD 20892.

Dopamine and excitatory amino acids play important roles in the basal ganglia control of behavioral circuits. The transmitters interact may provide new therapeutic approaches to the treatment of Parkinson's disease. We used the 2-deoxyglucose (2-DG) autoradiographic technique to examine the effect of an antagonist of D1 dopamine receptor dizocilpine (MK-801) on regional cerebral metabolic responses to D1 and D2 dopamine agonists in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway. The D1 agonist SKF 38393 (5 mg/kg, i.v.) increased glucose utilization in substantia nigra pars reticulata and entopeduncular nucleus. The D2 agonist quinpirole (1 mg/kg, i.v.) was without effect in these regions, but decreased 2-DG uptake in the nucleus accumbens. Pretreatment with MK-801 (0.1 mg/kg, i.v.), which had little effect on cerebral metabolism by itself, reduced the effect of SKF 38393 in both substantia nigra pars reticulata and entopeduncular nucleus and prevented the effect of quinpirole in the nucleus accumbens. Both SKF 38393 and quinpirole decreased glucose utilization in the lateral habenula. MK-801 pretreatment did not alter the effect of SKF 38393 in the lateral habenula but substantially reduced the effect of quinpirole in this structure. These results indicate that D1 and D2 receptor-regulated brain mechanisms are differentially influenced by NMDA receptor stimulation. D2-mediated cerebral metabolic responses appear to require concurrent NMDA receptor activation, while D1 receptors do not always exhibit varying degrees of sensitivity to NMDA receptor blockade.

503.12 IMMUNOCYTOCHEMICAL STUDY OF NONPHOSPHORYLATED NEUROFILAMENT PROTEIN DISTRIBUTION IN MONKEY BASAL GANGLIA. M. Taniguchi, T. Kondo, T. Nakazato, and T. Okabe, SMI-32 (Sternberger Monoclonal Inc.), is a monoclonal antibody that avidly recognizes the KSP segment of NF-H and weakly recognizes the KSP segment of NF-M in nonphosphorylated state (J. Neurosci. Res. 30:47). Unlike some NFP antibodies it lacks cross-reactivity to the microtubule associated proteins (PNAS 83: 1998, & PNAS 84: 3410). In studies of primate neocortex, SMI-32 labels primary dendrites and dendrites of a population of pyramidal neurons whose distribution differs markedly across cortical areas (e.g., J. Comp. Neurul. 282:191). This examination of the basal ganglia reveals striking differences between the striatal and pallidal compartments. The caudate and putamen are relatively unique brain regions in that there is an absence of immuno-reactive neurons. Rather, there is a dense staining of astrocytes which exhibits some regional variation (generally, the dorsal striatum is more densely stained than the ventral striatum). However, the KSP protein exhibits variations in intensity that correspond to both subcellular and matrix organization of the striatum as revealed by Aβ receptor like a) and a b) immunoreactivity for example (PNAS 75:2732 & PNAS 82:8780). In contrast in pallidal segments, a large subpopulation of neurons and their extensive dendrites are immunoreactive. These observations suggest that one or more of the terminal projections delineating a subcompartimentalization of the striatum also requires this form of NFP. Whereas, in the pallidum, as in the neocortex, it is a cytoskeletal specialization in the soma and dendrites of a large subpopulation of projection neurons, differences in neurotransmitter used by neurons in these two regions (GABA versus excitatory amino acids).
503.13

In the striatum, patchy compartments (the striosomes and their development forerunners, the dopamine islands) can be distinguished from the surrounding issue (the extrastriosomal matrix) by their differential content of numerous neurochemical compounds. The adenine-producing ectoenzyme 5'-nucleotidase (5N) is a new marker for striosomes in the mature rat caudoputamen, revealed with a histochemical lead technique (Schoen and Graybiel, Soc. Neurol. Abstr. 17, 452). On the basis of serial-section comparisons with the distributions of tyramine hydroxylase and calbindin-D28k immunoreactivity (marking dopamine islands and matrix), we show here that 5N is selectively enhanced in the neuropil of dopamine islands/striosomes of the rat caudoputamen from postnatal day 1 to adulthood. This holds for all but the caudal caudoputamen. In mouse, by contrast, 5N activity is associated with extrastriosomal matrix of the anterior and middle caudoputamen from embryonic day 18 until postnatal day 21; rarely, areas of enhanced 5N reaction product overlap with dorsally located dopamine islands. In adult mice, most of the caudoputamen is filled with dense histochemical reaction product, but rostral striosomes remain visible by low 5N activity. These results indicate a converse enzyme architecture of the caudoputamen in rat and mouse. As 5N is associated with glia and mantleable synapses, this enzyme could reflect specific glial compartmentalizations or sites of synaptic plasticity within striatum. Roles of 5N in purinergic neuronomodulation and cellular contact formation may contribute to activity-dependent or adhesive mechanisms underlying the differentiation and maintenance of the striosome and matrix compartments. Supported by NIH (ROI NS05259) and DFG.

503.14
GRADIENTS IN THE DopAMINE-REGULATED SYNTHESIS OF NEUROPEPTIDES AND Dopamine RECEPTORS IN RAT NUCLEUS ACCUMBENS. P. Vrooman and J.C. Driessen. Dept. of Anatomy and Embryology, Frye Univ., Amsterdam, the Netherlands.

Quantitative in situ hybridization histochemistry was used to study the effects of unilateral 6-hydroxydopamine lesions of the ascending dopaminergic fibers on the synthesis of enkephalin (Enk), dynorphin (Dyn), substance P (SP) and the dopamine D1 and D2 receptors in projection neurons in subregions of the nucleus accumbens (Acc) and in the caudate–putamen (CP). In CP of control animals a systematic increase of mRNA along the rostrocaudal axis was found for Dyn and D-1, whereas in Acc a decrease in mRNA was noted in the rostral to caudal direction for all three neuropeptides and the two dopamine receptors. Two weeks after the lesion an increase was found in Enk and D-2 mRNA, both in CP (Enk +106%, D-2 +36%) and in Acc (Enk +73%, D-2 +13%), in the lesioned side compared to the non-lesioned side. A decrease was observed for Dyn, SP and D-1, which was the same in CP and Acc for D-1 (-15%) and Dyn (-58%), and slightly higher for SP in CP (-44%) than in Acc (-39%). The adaptive changes in mRNA levels appeared to be proportional along the rostrocaudal axis for all neurotransmitters and receptors except for Enk. For Enk mRNA the increase in Acc was rostrally higher than caudally, indicating regional differences in the effects of blockade of the dopaminergic neurotransmission.

503.15
CHARACTERIZATION OF A NEW BRAIN-SPECIFIC PROTEIN TYROSINE PHOSPHATASE IN RAT STRIATUM AND CEREBRAL CORTEX. J. R. Nance, M. Leven, and P. I. Lombroso. Dept. of Biology, Wesleyan University, Middletown, CT 06457 and Child Study Center and the Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510.

Protein tyrosine phosphatases (PTPs) act in opposition to tyrosine kinases to regulate phosphorylation of tyrosine residues. These two classes of enzymes are implicated in cell differentiation but may have additional functions in mature cells. We have explored the possibility that different PTPs might be present within functionally distinct regions of the brain. A novel intracellular PTP was cloned from a rat striatal cDNA library (Lombroso et al., V. FNAS 86: 7242-7246). Northern analyses of brain revealed a 3 kb and a 4.4 kb mRNA. The 3 kb mRNA was highly enriched in the striatum relative to other brain regions and was termed striatal enriched phosphatase (STEP). An amino acid consensus sequence found in all protein tyrosine phosphatases and tyrosine phosphatase activity identified STEP as a PTP. Rabbit polyclonal antibodies against STEP recognized a major band of 46 kDa and minor bands of 37 and 33 kDa on Western blots of adult striatum. In sections of fixed rat brain, STEP antisera stained striatal neurons and neuropil. In cerebral cortex, a subset of neurons were stained in layers 2-3, 5, and 6. Staining was also found within the neuronal subsets in hippocampus and lateral septal nucleus. These findings suggest the existence of other brain specific PTPs showing regional heterogeneity.

503.16

Cholinergic interneurons and substance P/neurokinin A medium spiny projection neurons comprise 5% and 40%, respectively, of the total number of neurons in the rat caudate-putamen (CPa). Since the influence of cholinergic interneurons on neuropeptidergic projection systems in the striatum is poorly understood, this study explores the relationship between cholinergic receptor activation or inhibition on the tachykinin gene expression.

Adult male Sprague-Dawley rats were treated chronically either with a cholinergic agonist (physostigmine: 0.5mg/kg/days), or a muscarinic agonist (scopolamine HCl: 0.4mg/kg/days), or vehicle (PBS: 0.1ml/100g) administered for 6 days (s.c.). Rats were perfused with 3% paraformaldehyde and coronal cryostat sections (20µm) were cut and mounted on Vectabond (Vector) coated slides. In situ hybridization was performed with a full-length (560nt) ribonucleotide probe directed against P0-ppt (a transcript containing substance P, neurokinin A, and other tachykinins). Physostigmine administration resulted in a small decrease in tachykinin expression in the CPa and olfactory tubercle, while scopolamine treatment resulted in an increase in expression in both regions, as compared to vehicle treated animals. The increases after scopolamine treatment appeared to be greatest ventrolaterally in the CPa, paralleling the distribution of ChAT mRNA positive cells. The results suggest that acetylcholine in the striatum may regulate levels of tachykinin expression in a manner opposite to that observed with dopamine. Supported by NS24418.

503.17

Dopamine (DA) agonists have been shown to induce c-fos gene expression in the caudate-putamen (CPa) of rats. To test for a synergistic interaction between D1 and D2 receptors in DA-mediated c-fos expression, we injected neurologically intact rats with either the D1 agonist SKF 38393 (20 mg/kg), the D2 agonist quinpirole (3 mg/kg), the combination of both SKF 38393 and quinpirole, or saline. Two hours later, rats were perfused (4% paraformaldehyde) and the brains prepared for immunohistochemical visualization of Fos, the protein product of the c-fos gene using Cambridge Research Biochemicals antibodies OA-11-825 (1:1000). Striatal Fos immunoreactivity (Fos-Ir) was virtually undetectable in rats injected with saline, SKF 38393, or quinpirole. In rats given the combination of D1 or D2 agonists, however, patches of nuclear Fos-Ir were readily detectable, particularly in the posteror CPa. In a second experiment, rats were given daily injections of vehicle or reserpine (1 mg/kg), a treatment that results in a breakdown in D1/D2 synergism. On the fifth day, they were injected with either SKF 38393 (20 mg/kg) or saline and perfused 2 hours later. SKF 38393 alone elicited pronounced Fos expression homogeneously throughout the CPas of rats pre-treated with reserpine, but not vehicle. The 5-day regimen of reserpine treatment did not itself elicit Fos expression. These data suggest that DA-mediated c-fos expression is a histochemical indicator of the state of D1/D2 synergism.
504.1 DIFFERING CLIMBING FIBER LENGTH IMPLIES VARIABLE CONDUCTION VELOCITY TO ESTABLISH ISOCRHYONICITY OF CLIMBING FIBER CONDUCTION TIMES. L. Sugihara*, E. J. Lang, C. I. de Zeeuw and R. Linck. Dept. of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, N.Y. 10016.

Previously we demonstrated that the climbing fiber conduction times are tuned closely to the 9.5 to 14.0 mm in lobe crus 2a regions of path length from the inferior olivary (IO) to the cerebellar cortex, and that the rostro-caudal banding pattern of synchronous spontaneous complex spike (CS) activity in Purkinje cells stretches down the side of the cerebellar folia (Soc. Neurosc. Abs., 1980). We investigated this isocrhronicity further in ketamine-anesthetized adult rats. Conduction times for the other hemispheric and ventral lobules were also found to be about 4.0 ms (4.0 ± 0.4, mean ± SEM, n=600 cells). Although CS activity in the ventral and hemispheric PCs are not strongly correlated in control condition, systemic application of picrotoxin can increase synchronicity of their CS activity by increasing the electrotonic coupling between olivary cells. In that situation, the CS activity of ventral and hemispheric PCs, recorded simultaneously using a multi-electrode technique, are synchronous to within 3 ms as determined from the peak of the cross-correlograms. To investigate the basis of CS isocrhronicity, PHA-L injections into the IO were made to visualize the climbing fibers to the vermis and hemispheres. The coordinates of the center of the stained fiber bundles in 90 μm thick coronal sections were measured and reconstructed in a three-dimensional space. The length of the fiber bundles to the vermis (zone A) ranged from 9.5 to 14.0 mm while the bundles to the hemisphere (zone B) were shorter, 7.0 to 11.0 mm. These results further confirm the finding that the conduction velocity of a climbing fiber is correlated to its length so as to establish isocrhonic conduction times. Supported by NIH grant NS13742.

504.3 QUALITATIVE DYNAMICAL MODEL OF BISTABILITY IN PURKINJE CELL DENDRITICES. G. L. F. Yeung, P. E. Hockberger, L. E. Mason and J. C. Hoy. Dept. of Physiology, Northwestern University Medical Center, 303 E. Chicago Ave., Chicago, IL 60611-3000.

Previous computational and electrophysiological studies suggest that cerebellar Purkinje cells may function as bistable elements with hysteresis in the context of movement control (see Carter et al., this meeting). To develop more realistic models of bistability, this phase-plane analysis was carried out using recent whole cell data. In particular, combinations of calcium and potassium channels were introduced into a dendritic compartment (with rapid impedance load) and bistability of generating bistable instability was investigated.

Preliminary results from the use of high-threshold calcium and delayed rectifier potassium channels, which have been described in these neurons, suggest that they can support bistability in a dendritic compartment. In this model, the dynamical state variables are membrane potential and potassium channelactivation. Calcium activation is assumed to be instantaneous and a bifurcation function of membrane potential. The nullclines of the state variables have three intersections or equilibrium points, of which two are stable (representing excited and the rest state) and one is unstable. State transitions can be induced by either short or long current pulses. For example, a depolarizing current can send the system into the excited state (at a higher membrane potential) which it will remain at until a hyperpolarizing pulse returns it to the resting state. A larger stimulus was needed to effect the (excited-rest) transition compared to excitation (rest-excitation). Thus the voltage-current relationship exhibited bistability and hysteresis in that different stimulus thresholds were observed for excitation and recovery. These properties may allow Purkinje cells to participate in delayed feedback pathways without causing system instability.

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Both brainstem and floccular sites have been proposed as principal locations for plasticity resulting in vestibulo-ocular reflex (VOR) adaptation. However, specific predictions concerning the nature of the error signal required for the adaptation process have not been articulated. Here we explore the computational characteristics of simple neural network models of these brainstem and cerebellar loci by building a model based on the formalization of Mies and Lisberger (Ann.Rev. Neurosci., 1981, 4,273) and tested it's ability to generate normal VOR error signals in response to a variety of head velocity step inputs. Tuning the network using an unphysiological global optimization method indicated that the most accurate adaptation response occurs when synaptic weights are allowed to change at both sites.

Turning to more physiological learning rules, we found that using output of gaze-velocity Purkinje cells isn’t effective as a teacher signal for driving plasticity at brainstem locations. Rather, it leads to wildly oscillating behavior because this output is part of a positive feedback loop. Optimal adaptation occurred when retinal slip velocity, representing output of the accessory optic system, was used to directly instruct changes in vestibular input weights at brainstem and cerebellar sites using a Widrow-Hoff learning rule. Unsupervised Hebbian rules are inadequate and cause the system to adapt to inappropriate gains. This finding on learning rule efficacy confirms for a specific instance the general observations of Sutton and Barto (Psych.Rev., 1981,88,135). Supported by EY06485, EY03742, DCO1559.

504.6 A NEW TREATMENT OF SPATIO-TEMPORAL STRUCTURE IN ARRAY RECORDING OF THE RAT INFERIOR OLIVE. A. Sivaramakrishnan, E. J. Lang, I. Suphara, S. Sivaramakrishnan* & R. Linck. Department of Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York NY 10016 & Biology Department, 139-74, California Institute of Technology, Pasadena CA 91125.

Statistical tools developed for data with continuous or many-valued data are often inappropriate for binary data that may be the best representation of neural on/off states. We present an approach that incorporates a non-linear prefiltering of data from microelectrode array recordings of Purkinje cell complex spikes from the surface of the rat cerebellar cortex, followed by calculation of a correlation-type measure that is tailored to this type of data. This statistical approach appears to be better in many ways than the traditional forms of correlation. Data from these recordings are to be used to assess the role of oscillation and rostrocaudal banded patterns of Purkinje cell climbing fiber activation that seem to form the “grammar” of neuronal organization of the inferior olivary nucleus, the site of origin of the climbing fiber system. This statistical approach is robust in the sense that it is not affected by the considerable variation in the corresponding 100ms “periods” between spikes trains. The method shows structure in the data that is often washed out by the usual approaches of autocorrelation and cross-correlation. (NINCDS-13742)

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504.7
MICROCIRCUIT ASSOCIATIVE MEMORY MODEL OF THE CEREBELLAR CORTEX. C. E. Miles and M. A. Nudelman. St. George's Center and Department of Psychiatry, University of California, San Diego, CA 92103.

In this work, information and analytical techniques from the fields of biology, mathematics, computer science, and engineering are used to model the information processing characteristics of the cerebellar cortex. This approach is based on the idea that the cerebellar cortex is a highly interconnected network of neurons that can be described using a self-organizing map.

By viewing anatomically different neurons as representing network elements whose input-output functions differ, a connectionist hierarchical network model for distributing information throughout the memory is proposed. The functional circuitry developed to implement this feature is called the MicroCircuit. The MicroCircuit provides a means of combining sensory input signals from different modalities. Unique to the MicroCircuit is its use of interneuron associations to establish an inter-model communication network and a proposed functional role for climbing fiber activation during memory recall.

Interconnected MicroCircuits form the MicroCircuit Associative Memory (MCAM) architecture. This offers a distributed and robust reconstruction of stored patterns during memory read operations. Analog results describe the design and write operations, pattern fidelity, and immunity to noise. Computer simulations, using a 4096 processor Connection Machine, are used to verify our theoretical predictions.

504.8
REALISTIC COMPUTER SIMULATIONS OF MEDIAL VESTIBULAR AND DEEP CEREBELLAR NUCLEI. NEURONS. S. Quadroni, J. Simonet and G. Knopfel. T. T. Federal Institute of Technology, Dep. of Physics, CH-8093 Zurich and Brain Research Institute, University Hospital, CH-8092 Zurich.

We have developed a user-friendly program running on UNIX workstations for computer simulations of single nerve cells, involving an X-window-based editor permitting design, variation of morphological and membrane parameters. An integration routine based on an implicit second order algorithm allowed fast and stable integration of coupled differential equations describing the kinetics of ion-channels as well as concentrations, diffusion and scaling of Ca++. We have designed computational models of type A and B guinea pig medial vestibular nucleus neurons (Seraphin et al., Exp Brain Res. 84:417-433) and of rat deep cerebellar nucleus neurons. Compartment comprise up to eight active ionic conductances (I_{Na}, I_{K}, I_{Ca}, I_{KCa}, I_{G}, I_{GK}, I_{K}, I_{P}) and whose kinetics were obtained from voltage-clamp studies in a variety of preparations. Synaptic inputs of the AMPA, NMDA, and GABA_A type were simulated with appropriate changes in conductance. Some kinetic parameters such as distribution and density of ion-channels were modified to faithfully reproduce the responses of living neurons as revealed in current-clamp experiments.

HUMAN COGNITION II

505.1
BASIC INFORMATION TRANSFER DEFICITS ASSOCIATED WITH CLOSED HEAD INJURY. Does CALLOSUM DEFECTS CORRELATE WITH THE MEMORY FOR A TACTILE STIMULUS? CM Fernandez-Card* and R. D. Fantie, Human Neuropsychology Laboratory, The American University, Washington, DC.

Neuropsychological studies have consistently indicated there is disproportionately severe damage to the corpus callosum in closed head injury (CHI) and that this can occur when the trauma seems relatively minor. To determine whether we could detect callosal dysfunction in the inherent processing of tactile manual stimuli, we employed a basic information transfer test that required subjects to match small sets of pinheads utilizing only their sense of touch. Each person used either the same hand (Right-Right or Left-Left) or opposite hands (Right-Left and Left-Right) and was asked to match stimuli and find its matches from within a series of distractors. One phase of the experiment (Simultaneous) allowed participants to leave one hand on the model while the other hand was free to search in the hand condition, return to the model as often as desired. Another phase required that the subject search for matches from memory only (Successive). In the Simultaneous condition, the performance of the CHI group (n=10, mean age=27.4 years) did not differ significantly from that of Controls (n=20, mean age=24.8 years) although there appeared to be a trend for the CHI group to perform more poorly than Controls in the 2-hand condition. In the memory-dependent Successive condition, the CHI group was significantly slower and made more errors than Controls when searching for targets with the right hand. We propose that this pattern of results is compatible with a retrieval deficit resulting from a disconnection between the left hemisphere's motor control of the right hand and transcallosal access to the representation of the target stimulus presumably encoded spatially in the right hemisphere.

505.2
SPECIFICITY OF INHIBITORY TRANSFER FOLLOWING A PARTIAL LESION OF THE CORPUS CALLOSUM. S. B. Davies, M. J. Tramo, T. B. Fendrich, A. O. Reoves, and M. S. Gazzaniga, Program in Cognitive Neuroscience, Dartmouth Medical School, Hanover, NH 03755, T. Dept. of Neuropsychology, Harvard Medical School, Boston, MA, and TCenter for Neurobiology, UC Davis, Davis, CA.

Evidence is presented to demonstrate that the hemispheric transfer of a tactile task with intact visual transfer is disrupted only if the corpus callosum is divided. This transfer is mediated by the intact portion of the corpus callosum. These findings indicate that 1) somesthetic information within the right hemisphere could be used to perform unimanual and crossmodal intra- and interhemispheric transfer, and 2) transfer of somesthetic information within the right hemisphere to left hemisphere language areas was disrupted, and 3) different regions of the callosal mediate the transfer of somesthetic information about texture vs. tactility.

Supported by Javits Award NS12266 (MS), MH118012 (MJT) and DC08811 (KB).

505.3

In a recognition task in which 215 objects from varied conceptual categories were presented visually, a patient with a lesion in right parietal cortices (areas 5 and 7) performed at the same level as controls (98%). However, when the patient was blindedfolded and 100 such stimuli were presented in the tactile mode, to the left and right hands independently, there was a severe entity-selective impairment. When the stimuli were grouped into those that are manipulable and those that are not, we found that right- and left-hand recognition of manipulable entities was 94% and 97%, respectively (comparable to controls), while the performance on nonmanipulable items was only 57% and 47%, respectively (severely defective). Thus, the patient could nearly always retrieve the concept behind a manipulable entity, but was often unable to retrieve the concepts behind nonmanipulable entities. We propose that the lesion prevents signals from left and right somatosensory cortices from accessing right inferotemporal systems that are critical for a specific conceptual structure of visually ambiguous, nonmanipulable entities. However, such signals can still access the motor system through a frontal route that taps part of the conceptual structure for manipulable entities.

505.4

The stereotypic, species-specific character of laughter facilitates the analysis of the neurobehavioral mechanisms for the production, perception and evolution of human auditory signals of which speech is a special case (Eiholzer, 89 (1991) 115-124). Laughter and speech are seldom considered in the same context, a tradition that limits our understanding of both behaviors. This study describes the position of naturally-occurring laughter in the speech stream of anonymous, mostly adult subjects, observed in public places. Laughter of both speaker and audience occurred during pauses at the end of sentences in over 99% of the sample of 1200 episodes of laughter, indicating that speech has priority access to the vocalization channel and that a lawful neurobehavioral process governs the placement of laughter in the speech stream. Laughter by speaker or audience rarely interrupted the phrase structure of speech. Because laughter followed both statements (84.3%) and questions (15.7%), it was not restricted to a specific type of preceding sentence. Contrary to experience with professional comedians, most laughter was not preceded by jokes or material that seemed humorous outside of the conversational context. Another counterintuitive finding was that speakers laughed more than audiences. Speakers, especially females, laughed more than their audiences, but the relative amount of speaker and audience laughter depended on the gender composition of a group. Audiences of both males and females laughed more than their speaker, or by the other, or in the hands of a speaker. It is how the laughter may be why most professional comedians are male. These baseline data define the variables for future studies of laughter in neuro- and psychopathology. Laughter may provide novel insights into the neurobehavioral mechanisms of normal and abnormal emotional communication in humans.
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HUMAN COGNITION II

THURSDAY AM

505.5 ELEMENTS OF ATTENTION: PERFORMANCE OF HEALTHY ADULTS AND NEUROPSYCHIATRIC PATIENTS ON THE NIMH-LFP ATTENTION BATTERY. J.E. Taima*, R.D. Fantie†‡* and A.E. Minsky‡. National Institute of Mental Health, Bethesda, MD.

Healthy adults (n=70), aged 18 to 90 years, performed the NIMH-LFP Attention Battery; a set of neuropsychological tests tapping a broad range of attentional abilities. Extending and refining the four elements of attention that we identified in the battery (i.e., 1. Focus/Execute, 2. Shift, 3-Sustain, and 4-Encode), a new five-component solution best resolved the variance in these data. We derived the newly-identified factor from variance and error scores on the Continuous Performance Task (i.e., 5-Reliability of Performance) and renamed the original 4 factors 1-Scan/Focus Speed, 2-Flexibility/Shift of Set, 3-Arousal/Effort, and 4-Encode/Retain, respectively. A reanalysis of a subset of Minsky et al.'s data resulted in a comparable 5-component solution among 52 neuropsychiatric patients (i.e., patients with petit mal and complex partial seizure disorders, anorexia nervosa and bulimia nervosa, affective disorders, and closed head injuries), aged 20 to 63. Despite interesting differences, we argue that the underlying components reflect congruent cognitive processes in each group. We also propose that the disordered attention in these clinical subjects may be primarily attributable to poor executive regulation of attentional shifts, i.e., an impaired flexibility of cognitive set. In summary, we obtained general support for the reliability of Minsky et al.'s original four elements while the new 5-component solutions cast several new lights on the performance of both healthy and neuropsychiatric subjects on tests of attention.

505.7 AUDITORY STREAMING REDUCES REACTION TIMES TO INFREQUENT TARGETS. C. Alain* and J.L. Woods. Clinical Neuropsychology Laboratory, Dept of Neurology, UC Davis, VA Medical Center, Martinez, CA 94553, USA.

When a sequence of different tones is presented rapidly, the sequence may split into two or more perceptually concurrent streams. The tones that are similar in frequency will end up being perceived as a group. This phenomenon is called "auditory stream segregation" or "streaming" and is sensitive to stimulus rate and tonal separation. In two experiments we examined whether streaming would affect reaction times as well as other measures of auditory perception. Stimulus sequences consisted of tones (40 ms, 78 dB SPL) of three different pitches presented in random order. Tones were delivered over headphones at either fixed (100 ms) or variable interstimulus intervals (ISI 140 to 220 ms). In the "baseline" condition, the three pitches were evenly spaced in frequency (by six semitones, 1048, 1482, 2096 Hz). Subjects responded to infrequent (2.5%) longer duration tones (65 ms, Exp. 1) or infrequent louder tones (85 dB SPL, Exp. 2) of a designated extreme pitch (1048 or 2096 Hz). In the "grouping" condition, the middle pitch was unchanged (1482 Hz) whereas the low or high pitched tones were made more similar (by one semitone) to tones of the middle pitch. It was hypothesized that the increased proportion of the irrelevant pitches would segregate them into a separate stream, thereby facilitating the monitoring of the target belonging to the relevant pitch. In both experiments, tonal grouping increased the number of correct responses and decreased RTs compared to the ungrouped condition. This grouping effect on RT was similar at a fixed or variable ISI. This result suggests that stream segregation is a general phenomenon that influences the speed as well as the accuracy of auditory identification.

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In three separate experiments, subjects were required to attend to either their right or left ear in a dichotic listening task, with target ID task. In Experiment one, subjects simultaneously were required to perform a verbal task in which word pairs were centrally presented on a computer screen. The words were to be identified as either antonyms or unrelated. In Experiment two, subjects simultaneously had to perform a mental rotation task in which two histograms were presented successively and had to be identified as being identical or mirror images. In Experiment three, subjects simultaneously performed a two dimensional tracking task in which two cursors had to be kept in the center of the video screen.

Results of Experiment one indicated that, for male subjects, attending to the right ear interfered with performance of the verbal task more than attending to the left ear. Ear attention did not differentially effect female performance. For Experiments two and three, for females, left ear attention interfered with performance on the spatial tasks more than right ear attention. Male attention did not effect spatial tasks.

505.9 NONTRIVIAL SEX DIFFERENCES IN BRAIN WEIGHT AND CRANIAL CAPACITY CONTROLLING FOR BODY SIZE. J.M. Depue, M.A. Pelchat, Psychology, University of Western Ontario, London, Ontario, N6A 5C2, Canada.

Although it has long been known that human females have absolutely smaller brains (mass or volume) than do human males, it is widely thought that they would be equally capable of academic achievement. We wondered if the differences disappear. Two large data sets show that after covariance adjustment for body size, women's brains average 100 g lighter and 11 cm smaller than men's.

First, Ankeny (in press, Intelligence) reanalyzed and published in 1980 on 1,261 subjects between the ages of 25 and 80. Second, Rushton (in press, Intelligence) analyzed and published in 1988 the combined data from 9,192 subjects in four large, stratified random samples across White and Black men and women. The data from across these two sets is replicated across White and Black samples in the first study and across Asian, White, and Black samples in the second. These differences may be related to human evolution and those intellectual abilities at which males excel.

We previously demonstrated impairment of sleep-wake and temperature rhythms, and temporal attention dynamics in a human (AH) following SCN region destruction. We have now studied AH's short-duration timing. Severe disruptions of motor timing and time estimation compared to a age-matched control. A capillary continuation paradigm was employed. The Wing & Kristofferson model was used to derive clock and motor-delay variance. AH exhibited greater clock variances than previously reported after cerebellar lesions. AH's clock variances increased with task, indicating that short-duration timing was greatly influenced by attentional biases. These results indicate that other neural systems, in addition to the cerebellum, may influence short-duration timing and also suggest a hierarchical role for the SCN in such timing.


Is a recent study (Lassonde et al., B&L, 1991), we reported that language functions, as assessed by dichotic listening performance, were not strongly lateralized in callossal agnosia subjects than in IQ-matched normal controls. However, the task used (CV detection) was so demanding that it produced a performance advantage in some subjects. The present study was thus designed to verify whether the single-motor effect found in callossalcs could be observed regardless of task difficulty. Four adult callossalcs and four IQ-matched controls were tested in two simple dichotic tasks. The tasks consisted of word and musical timbre detection and were devised such that they would yield both lateral effects and high accuracy levels. The two tests were also presented monaurally since this easier procedure has occasionally been shown to produce lateral effects. In terms of accuracy, the two groups performed equally well, both monaural and dichotic presentations (average performance: 96% correct). Analysis of response times, however, revealed different patterns for the two groups in the dichotic and monaural conditions. In the dichotic condition, controls as well as callossalcs, showed reliable lateral effects. In the monaural condition, controls did not demonstrate any ear advantage while callossalcs still exhibited ear advantages of the same magnitude as in the dichotic condition. These findings suggest that callossal absence may weaken, rather than reinforce, the influence of ipsilateral auditory pathways. The results further suggest that the ipsilateral suppression normally observed under dichotic presentation is not related to callosal inhibition.


A.R. contracted a viral encephalitis at age 9. She was evaluated over a period of 4 years. CT scan revealed a right temporal hypodensity affecting gray and white matter and hypodense sub-cortical zones in the left temporal and right parieto-occipital areas. A.R. showed evidence of associative visual agnosia, prosopagnosia and color agnosia. At first, she was unable to name objects from visual inspection but could do so by verbal description, tactile and auditory cues. Moreover, she could not recognize familiar faces or identity colors. With time, A.R. has shown limited improvement. She can now recognize familiar objects although occasionally making intra-categorical mistakes. She can also name colors but cannot associate them to objects. Her prosopagnosia has not resolved. In fact, while she can match and copy visual representations of objects, she cannot do so in the case of faces. A clear dissociation between object and spatial representations is present in this patient. A.R. is unable to draw an object from memory but she can draw a map of familiar routes. She cannot correctly reproduce shapes that she has manipulated on a board but can accurately reproduce their localization. A.R.'s inability to recognize objects is interpreted as reflecting a deficit in the inferior temporal ventral pathway specialized for object perception, whereas the posterior parietal dorsal pathway specialized for spatial perception remains functional. Finally, the little copy displayed by A.R. indicates limits in cerebral plasticity for visual agnosia.


Lateral inhibition (LI) occurs when repeated exposure to a non-attended stimulus inhibits later learning of associations to that stimulus. LI is an indirect measure of selective attention. Interest in the measurement of LI in humans has increased because of the finding that LI is reduced in acutely psychotic patients, and that LI in rats is sensitive to drugs that are dopamine agonists and antagonists. LI paradigms are limited in that they measure across groups of subjects and are difficult to repeat because of the learning effects of the pre-exposures and the consequent associations. A continuous performance task for LI measurement that overcomes some of these limitations has been developed through use of pilot studies. The task is divided into four phases. Subjects are instructed to watch a monitor and press a button each time a target is presented. Stimuli appear for 1 second with a 2.5 second inter-stimulus interval. During phases 1 and 3 20% of the stimuli are targets, 60% are letters, and 20% are a non-letter preexposure stimuli. During phase 2, LI always cues the target. The task begins with 100 trials to baseline the subject. The subject then proceeds to phase 2. The subject then proceeds to phase 3. A subject proceeds to phase 4. A subject experiences LI when the reaction time in phase 4 is slower than in phase 1. Pilot work shows that normal controls show LI in aggregate (p<0.01), and individually in 60% of normal controls at the 0.05 significance level. LI is robust after 3 weeks of repetitive but learning effects appear to be consistent with those of the first experiment. LI is the fourth report. (Supported by MH-57053)
505.17
HOMOSEXUALITY, COGNITIVE ABILITIES AND THE ORGANIZATIONAL HYPOTHESIS. J. A. Hall* and D. Kimura. Dept. of Psychology, Univ. of Western Ontario, London, Canada, N6A 5C2

Previous research has suggested that, due to potential differences in pre- and/or perinatal levels of sex hormones, homosexual males may possess patterns of brain organization and cognitive abilities which fall intermediate between those of heterosexual males and females. The performance of 17 homosexual male undergraduates was compared to a heterosexual male control group (N=60) on spatial and verbal tasks which reliably show sex differences. Unlike previous studies, homosexual males showed no significant reduction on a task of spatial rotation; however, they were significantly outperformed by heterosexual males on a practical throw-to-target task. Additionally, a significant gay male advantage on an ideational fluency task and a significant non-gay advantage on a test of mathematical problem solving were found. Testosterone levels, assayed from saliva, showed no difference between gay and non-gay males. These data do not support the idea that homosexual males have ability profiles which are simply intermediate between heterosexual male and female extremes.

505.19
TYROSINE REVERSES A COLD-STRESS-INDUCED MEMORY DEFICIT IN HUMANS. D. Shurtleff†, J.R. Thomas, J. Schrot, K. Kowalski, R. Harford, M.O. Thornton, P.A. Shea and M. Malik. Naval Medical Research Institute, Bethesda, MD 20889-5055

Eight male subjects performed a delayed matching-to-sample (DMS) task at an ambient temperature of 4°C (cold) or 22°C two hours after ingesting the catecholamine precursor tyrosine (150 mg/kg) or placebo, administered double-blind. The DMS task required a correct recognition of two simultaneously presented matrices, one of which had been presented as a sample matrix 2, 8 or 16 sec before. Each matrix was composed of 32 red and 32 green squares, randomly distributed. After ingesting placebo at 22°C, subjects demonstrated a characteristic delay gradient in which accuracy declined as the delay interval increased between sample and comparison stimuli from 2 (Mean percent correct=83.76% ± SEM=1.38) to 8 (80.21% ± 1.66) to 16 (69.31% ± 2.50) sec. A one hour cold exposure following placebo ingestion significantly reduced matching accuracy at the 16-sec delay interval (50.41% ± 2.06), which is attributed to cold’s effect on short-term, or working, memory. Administration of tyrosine significantly improved matching accuracy at the delay interval affected by cold exposure (16-sec), such that mean matching accuracy was 65.76% ± 2.8, equal to that at 22°C following placebo ingestion. Plasma norepinephrine, epinephrine and tyrosine levels during cold and 22°C exposure were also measured and will be presented. These results indicate that tyrosine was effective in ameliorating cold stress effects on DMS performance, possibly by preventing a cold-stress-induced reduction in brain catecholamine levels.

505.20
DYNAMICS OF MODULATED NOISY BIOLOGICAL SYSTEMS. D.R. Chialvo, C.J. Hodges, and A.V. Aukan. Computational Neuroscience Program, Dept. of Neurosurgery, University of Health Sciences Center, Syracuse, New York 13219

The output signal from noisy bistable systems can be modulated in time by applying a weak external force. In this system, when driven by noise with power spectrum (ISIH) of a sine wave with an exponential envelope. For certain values of the noise and signal, bistable systems exhibit an increase in signal-to-noise ratio with an increase in input noise (stochastic resonance). Here we present: 1. numerical simulation of the mean response function and first few experiments, 3. a visual perception task, to show that the theory of bistable systems can be applied to information processing in the brain.

1. A two dimensional difference equation model of excitatory tissue driven with white noise results in spike trains with a Poisson ISIH. For a fixed noise amplitude, increasing the amplitude of an additional sine wave input results in spike trains with multipeaked ISIHs which become single peaked at higher sinusoidal amplitudes.

2. In anesthetized cats, single unit responses were studied in the spinal cord and the somatosensory cortex. Rapidly adapting type neurons showed multipeaked ISIH during sinusoidal vibrotactile stimulation of the receptive field. The envelope of these ISIHs had an exponential decay which increased with stimulus amplitude.

3. A cognitive visual task was developed to study hysteresis in viewing ambiguous figures. Subjects were familiarized with and tested to rank images consisting of a smooth transition from the face of a man to the body of a woman. The input/output map for ranking random sequences of these images showed hysteresis and was modeled as a 2-D linear map. When the images were presented iteratively with underlying noise and periodic modulation, increases in noise amplitude increased the perceptual signal-to-noise ratio, exhibiting bistable stochastic resonance. This paradigm can be used to study short term memory.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—MODELS

506.1
NEUROPOPULATIONAL MECHANISM OF PARALLEL CORTICAL PROCESSING IN THE MILLISECOND RANGE. K. Nakamura, K. Doi and T. Endo. School of Sci. & Eng., Tokyo Inst. of Tech., 4259 Nagataga, Yokohama 227, Japan

The cerebral cortex is capable of processing sensory signals to work the motor system in a few hundred milliseconds, though single neurons relatively slowly respond (several milliseconds). A mathematical model of the cortical processing is presented to show the processing is performed in an optimal parallel style. The cortex is represented by a number of areas consisting of various columns. Each column includes two populations of pyramidal cells and interneurons inhibiting the cells. Membrane characteristics of the model neurons is represented by the Hodgkin-Huxley electric circuit. Analysis of the model shows (1) the columns are capable of detecting several milliseconds with ratios of firing cells in the populations, even though firing of single cells fluctuates, (2) the areas perform complimentary functions of the normal mammalian cortex, where only the first activated columns are allowed to fire out of the columns activated in parallel, and (3) synaptic plasticity regulated by the hypothalamic reward system reinforces association connections between the brain's cortical center and the reward system centers leading to rewarding move of muscles. (1) and (2) indicate processing of each area completes in several milliseconds. This and (3) suggest repetitive rewards reinforce the whole cortex to respond to sensory stimuli within a few milliseconds.

506.2
EXPECTATION LEARNING IN THE BRAIN USING DIFFUSE ASCENDING PROJECTIONS. S. R. Quart*, P. Daron, P.R. Montague, T. J. Sejnowski, The Salk Institute, La Jolla, CA, 92037

Diffuse projections originating in subcortical nuclei are known to influence activity-dependent cortical plasticity and learning both during and after development. These signals may report to the cortex important events in the world as well as what activity patterns in the cortex result from actions taken by an organism e.g. proprioceptive signals associated with a movement. Although the number of physiological actions have been attributed to the neurotransmitters used by these pathways (acetylcholine, norepinephrine, serotonin, etc.), their precise functional effects on learning and memory remains unknown. We explore here a theory in which the derivative of the activity of the ascending pathways drives learning at cortical synapses. This learning is gated locally by a rapid diffusing signal produced by glutamate transmission in a local volume of tissue. We call this effect volume learning. This scheme forces the cortical networks to predict the future changes in activity in the ascending pathways. Moreover, a powerful influence driving cortical learning occurs when one part of the cortex captures control over the mid brain structure that releases the neurotransmitter. This occurs by allowing for multiple NMDA synapses on the path from cortex to the midbrain nuclei. Using a large scale computational model we demonstrate the theory and test it in a variety of tasks including visual recognition. This scheme may result in improved cortical representations in the absence of some way by which cortical patterns can be combined, decoded, and redistributed to the cortex. We show how the hippocampus can play a natural role in restructuring representations to make this prediction possible.

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506.3 HOW PLACE-CELLS CONNECTED TO HEBBIAN SYNAPSES CAN SOLVE SPATIAL PROBLEMS. R. Squire, R.U. Muller* and J.L. Kubie Dept. of Anatomy and Physiology, SUNY, Brooklyn. Brooklyn, N.Y. 11203

If place cells are connected by Hebbian (e.g., LTP-modifiable) synapses, the strength of the synapse between a pair of cells will come to encode the distance between their firing fields of the two cells. Synapses between cells with overlapping fields will strengthen because the two cells will fire together at intervals shorter than the LTP induction period. The overall strength of the synapse between cells with separated firing fields will remain weak because the two cells never fire in close temporal order. In this preliminary approach, such synapses do not affect the firing of the post-synaptic cells, they only register the degree to which the two cells fire together in time. If strengthening or weakening is added to a network that models the recurrent connections of CA3 (Traub and Miles, 1991), the result is an environmental representation that has the properties of a weighted graph. If a starting point and a specific point in real space are specified, a searching algorithm can be used on the graph to find the shortest path in reciprocal synaptic weight space. The sequence of place cells on this path corresponds to a path through real space. The length of the path in real space is very long if the network is small or sparsely connected. As the network becomes richer, however, the algorithmically computed path converges on the Euclidean distance between the starting and end points. The size and connectivity of adequate networks compare reasonably to the real thing. We conclude that graph-like networks of the proposed type contain enough information to make it possible to compute the shortest distance between any pair of points in space, thereby fulfilling many of the requirements for the hippocampal mapping system first proposed by O’Keefe and his colleagues.

506.4 INFORMATION PROCESSING IN EXCITATORY DENDRITES. B. W. Meister, Computation and Neural Systems Program, Caltech, 216-76, Pasadena, CA, 91125

Compartamental modeling studies of the input-output behavior of NMDA-rich neuronal pyramidal cells have previously shown that their dendritic trees were "clustering sensitive," i.e., gave rise to larger cell responses when synapses were activated in clusters, rather than by diffusely scattered about the dendritic arbor. This work has been extended to cells containing fast sodium and slow calcium spiking mechanisms in various distributions throughout the dendritic tree. Results indicate that strong cluster sensitivity can result when the dendrites contain 1) only NMDA channels, 2) only fast spiking channels, 3) only slow-spiking channels, or 4) combinations of any two or three of these voltage-dependent mechanisms in a variety of spatial distributions.

A model neuron called a "clusteron" was used to abstract the clustering sensitive behavior of a dendritic tree containing excitatory membrane mechanisms, and to explore a family of Hebb-type synaptic learning rules capable of manipulating the ordering of different synaptic connections onto the dendritic arbor. It is shown that the dendritic tree of a single pyramidal cell, if richly endowed with excitatory voltage-dependent nonlinearities, is capable of reliably discriminating thousands of learned complex patterns from unfamiliar controls. It is also shown that a cluster sensitive dendritic tree can perform a cross-correlation operation, relevant to biologically significant nonlinear sensory processes such as binaural disparity selectivity.

506.5 SIMULATIONS OF ADAPTIVE INTERACTIONS BETWEEN LIMBIC AND NEOCORtical STRUCTURES. S. D. Murphy and E. W. Kairat*, Dept. of Psychology and The Interdepartmental Neuroscience Program, Yale University, Box 208292, New Haven, CT 06520

Current views of long-term memory storage in mammals emphasize the importance of dynamic interplay between limbic structures (amygdala and hippocampus) and prefrontal cortex (e.g., L. Squire and S. Zola-Morgan, Science, 253:1380, 1990). The goals of this study were to construct a computational model that incorporates selected features of temporal lobe physiology and anatomy, and to examine the dynamical behavior of the adaptive processes that might support memory formation.

Hippocampal subfields (fascia dentata, CA3, CA1) were represented as networks of compartmental neurons, whose physiological properties, statistical connectivity and principle vulnerabilities were abstracted from physiological studies. A simplified cortical region was constructed as a multiple network of cortical pyramidal and non-pyramidal neurons; this network was reciprocally connected with the hippocampal networks. Input patterns were applied to the cortical and hippocampal structures in tandem, and stable cortical activity patterns were assumed to be as measures of information storage. Our studies focused on two properties thought to be important for mnemonic function: use-dependent synaptic plasticity (particularly at the hippocam-pocortical connections) and the role of theta rhythm in synchronizing connected systems. The spatio-temporal interactions between these phenomena were found to be important for the emergence of dynamic assemblies in the cortical network.

These computational studies will provide a framework for future analytical and experimental investigations of the dynamic processes underlying distributed memory storage in cortical systems.


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Given its placement on the olfactory-hippocampal circuit and unique anatomical features, it has been hypothesized that field CA3 of hippocampus maintains cue-specific activity after the cue is gone (and behavior continues) by generating recurrent and specific patterns of activity set in motion by perisynaptic path input, thus providing a short term memory system that can be used to form associations between cues that are separated in time and space.

To explore this hypothesis, we have constructed a computer model that incorporates many salient anatomical and physiological features of CA3. CA3 has a large population of pyramidal cells and is unique in its extremely dense recurrent, associational circuitry, which comprises >70% of the synaptic population (ipsilateral and contralateral). A smaller population of interneurons mediates fast and slow inhibitory currents. The model consists of 300 pyramidal cells, moosy fiber inputs, and a collection of interneurons. Total connectivity is less than 10% and is divided into global (random across the network) and local (random but spatially restricted) patterns for the various kinds of cells. Cells are modeled as simple compartments[1] incorporating driving force effects; multiple synaptic currents overlapping in time, neural delays and frequency facilitation. Baseline theta activity (30Hz) is generated by intrinsic input and on its own does not reliably trigger pyramidal cells.

Preliminary results indicate that recurrent patterns of activity can be generated in this network. Performance of the network and the network scaling was expected to improve performance. Thus, in principle, salient anatomical and physiological features of CA3 can be integrated to form a reverberating short term memory system. (Supported by grants from ONR and Matsushita Electric Industrial Co., Ltd.)


506.7 SEQUENTIAL CONFIGURATION MODEL AND TRACE DISRUPTION IN LOCAL, NEURAL NETWORKS. K.A. Flash†, R.J. MacGregor*†, G.A. Gadek‡, Dept. of Anatomy and Pharmacology, University of Colorado, Boulder, Colorado, 80309, †Dept. of Psychology, Denver, University of Colorado Health Sciences Center, Denver, CO 80022

The sequential configurations model is an explicit model of coordinated unit firing patterns for representing information in neural populations. A sequential configuration is an ordered sequence of sets of cells whose temporal firing relationships define the pattern. This work applies the sequential configuration model to represent memory storage and retrieval in two interconnected and recurrently connected populations of cells, one excitatory and one inhibitory, representing the various modules. Individual traces can be selectively recalled by specific stimulation of the network. The recurrent connections inherent in the trace produce internally sustained recasts of the sequential configurations. A theoretical derivation of memory capacity has shown that the level of cross talk can limit the number of traces that can be successfully recalled. The cross talk raises the average cell potential, producing a burst of activity. This burst causes many cells in the trace to be refractory at the same time, and produces simultaneous activation of a large population of inhibitory cells. This renders the trace inaccessible.


506.8 A MODEL OF FRAGMENTED SPATIAL MEMORY. Catherine Thivin-Blanc* & Marielle Kriem*. Cognitive Neuroscience Laboratory, CNRS, 31, ch. J. Aiguié 13402 Marseille Cedex 9 France. Tel: 91 16 40 88

Although the old opposition between behaviorists and cognitivists is now obsolete, the dual conception of two extreme and sometimes mutually exclusive forms of spatial memory is still alive. We propose another conception based on several inter-related levels of processing of a common "material", namely frontal local views whereby any terrestrial vertebrate, including human, has visual access to the environment. Several of these views (e.g., maps), stored in long-term memory, are the building-blocks of different forms of spatial knowledge.

This conception calls for several remarks. First, topographical maps are endowed with a definite spatial function supported by data from studies of unsighted adult subjects. Second, since topographical maps are assumed to be processed in different ways by the same individual, their storage and handling should be executed by distinct brain structures. In line with recent hypotheses, the topographical maps would be stored in visual areas whereas associative structures such as the hippocampus would be the repository of their "combinatorial" contents.

This model leads to several hypotheses and predictions which will be evoked.
506.9
PHYSIOLOGICAL MEASUREMENTS PREDICT THE LIFETIME FOR HUMAN AUDITORY MEMORY OF A TONE

Using the Magnetic Source Imaging (MSI) technique, we found that responses to tone stimuli observed in the primary and association areas of human auditory cortex provide evidence that the neural activation trace established by a stimulus decays exponentially with time, and the lifetime in association cortex is significantly longer than that in primary cortex in individual subjects. The strength of the activation trace at a time t following a stimulus was defined as the difference between the averaged response amplitude for a very long interstimulus interval and the averaged response amplitude for a given interval t. The difference was found to decrease exponentially according to exp(-t/T), where T is identified as the lifetime of the cortical activation trace. Behavioral measurements on the same subjects reveal that the short-term loss of auditory memory shows a central tendency, namely, the internal representation of the loudness of a sound evolves as a decaying exponential toward the mean of all stimuli experienced in the recent past. The time course is well predicted from the MSI measurements of the trace of activation in humanprimary auditory cortex. This close agreement suggests that primary auditory cortex plays a significant role in echoic memory. This application of MSI and behavior studies opens new opportunities to relate human physiology and cognition.

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507.1
HUMANS, LIKE RATS, HAVE SPATIAL WORKING MEMORY CAPACITY GREATER THAN SHORT-TERM MEMORY CAPACITY OF 7±2. R.B. Glassman* and R.C. O'Connor. Dept. of Psychology, Lake Forest College, Lake Forest, IL 60045.

Although humans show a short-term memory capacity for a wide variety of tasks (Miller, Psychol. Rev., 1956; recent review: Baddeley, Science, 1992), rats have a working memory capacity of 5±2 in the radial maze (Olton, Collison, & Werz, Learn. Motiv., 1977). Is this a species or situational difference? Using a 17-arm radial maze, we showed that on paper, human subjects attempted to lift in random order each of 17 cardboard flaps arranged radially around a center. Fiftteen undergraduates, each tested only 14 trials (each trial limited to 17 choices) averaged 15.4 correct responses. This suggests memory capacity comparable to rats. Miller's (1956) discussion of information load in absolute judgments implies a reason for greater spatial working memory capacity, in the two-dimensionality of the radial maze by comparison with the "linearity" of verbal sequences. Our finding presents a difficulty for the hypothesis that STM is a neurocognitive constant, based on widespread characteristics of mammalian cortex (Glassman, Neurosci. Abs., 1991).

507.2
ATTENTIONAL DEFICITS IN KORSAKOFF'S DISEASE. H.L. Young* & R.G. Mair; University of New Hampshire, Department of Psychology, Durham, NH 03824

Response times were measured for Korsakoff and alcoholic control subjects to execute keypress responses following presentation of a visual target preceded by a cue. Korsakoff patients responded more slowly in all conditions, however, their performances were significantly more impaired when cue and target occurred in different locations and when endogenous processing was required to interpret a cue. Comparable results were obtained when cues were not presented, but were expected in a location different from the target. We argue that the Korsakoff patients are impaired in their ability to disengage attention and shift it to a spatially distinct location.

507.3

Numerous animal studies have suggested a role for the prefrontal cortex in working memory processes. We developed a computer-administered human analogue of a spatial delayed response (SDR) task in order to study patterns of blood flow activation associated with the cognitive demands of this task. Subjects were required to remember the locations of 4 identical stimuli within an array of 20 possible locations, over a delay of 7 seconds. Patterns of regional cerebral blood flow (rCBF) during this task were compared to rCBF during a no-delay control task. Fourteen normal volunteers were studied using a H2O water technique. The results of this study support the hypothesis that the prefrontal cortex is involved in the cross-temporal maintenance of response-relevant information.

507.4

Sensor-motor skill learning has been dissociated from explicit memory in global amnesia and from repetition priming in Alzheimer's disease (AD). Patients with Huntington's disease (HD) learn poorly on some sensor-motor skill learning tasks, and these results suggest a critical role for the frontal-striatal system in these tasks. The present study examined whether two different kinds of sensor-motor skill learning, rotary pursuit and mirror tracing, are neurologically dissociable from one another. The subjects were 6 early-stage HD patients and 6 normal control (NC) subjects. For the rotary pursuit task, speed of rotation was adjusted for all subjects to equalize initial levels of performance, and subjects performed 4 20-second trials in each of 6 blocks. The main measure of learning was time on target. For the mirror tracing task, subjects traced a star seen in a mirror for 6 trials. The measure of learning was time to trace the star and the number of departures from the star. The HD patients showed impaired learning on the rotary pursuit task, but not on the mirror tracing task. These results suggest that there are at least two dissociable components of sensor-motor skill learning, and that one component, critical for rotary pursuit learning, is mediated by the frontal-striatal system. Preliminary data from two patients with cerebellar lesions suggest that another component, critical for mirror tracing, is mediated by a cerebellar system. We report a grant from the McDonnell-Pew Cognitive Neuroscience Program.

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507.5 INHIBITED WORKING MEMORY IN UNMEDIATED ADULTS WITH GILLES DE LA TOURETTE'S SYNDROME. G.T. Stebbins*, J. Singh, J.D.E. Gabrieli, C.L. Comella, and C.G. Goetz; Department of Neurological Sciences, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612 and Department of Psychology, Stanford University, Stanford, CA 94305
We assessed memory performance (including working memory (WM), immediate and delayed memory, and memory consolidation) in a sample of 13 unmedicated adult GTs patients, and 10 healthy controls (HC) matched for sex, mean age and education. WM was assessed using a verbal working memory task (Birdhouse & Balota, 1990) which required the subject to answer questions about orally presented sentences, while simultaneously remembering the last word in the sentences. Immediate memory was tested with forward and backward word span tasks. Long-term memory was assessed with free-recall and forced-choice recognition of word lists, and vocabulary knowledge (WAIS-IV Vocabulary subtest). Motor skill learning was assessed with the memory pursuit task. GTs patients were impaired on working memory (p<0.01), word span backwards (p<0.005), long-term free recall (p<0.05), and rotary pursuit learning (p<0.005). In contrast, GTs and HC performed comparably on long-term recognition memory (forced-choice recognition), long-term semantic memory (WAIS-IV Vocabulary subtest), and one test assessing immediate memory (word span forward). This pattern of memory performance is similar to that seen in diseases of the basal-ganglia (Parkinson's disease and Huntington's chorea), and suggests that the memory deficits in GTs may be due to selective frontal-atrietal dysfunction.
Supported by the Tourette Syndrome Association.

507.7 INFLUENCE OF FRONTAL LOBE LESIONS ON SUBJECTIVE ORGANIZATION IN VERBAL LEARNING. P.J. Elinge* & L.M. Grattan, Deps. of Neurology, Penn State Univ. Coll. of Medicine, Hershey, PA 17033, & Univ. of Maryland Medical School, Baltimore, MD 21201.
Subjective organization (SO) refers to the extent to which subjects spontaneously impose a sequential structure on the free recall of unrelated words. It is well established that SO is important to efficient learning and memory in normals; however, the neuroanatomical underpinnings of this process are minimally known. In light of recent findings implicating the frontal lobes in spatial-temporal processing, we hypothesized that frontal lobe lesions may cause a disproportionate deficit in subject-imposed sequential organization. To investigate this possibility, we compared the SO of 30 patients with stable, focal lesions to frontal and non-frontal cerebral regions. Using a free recall paradigm, each subject was administered a 15 word stimulus list for 5 learning trials. We tabulated the extent to which each subject imposed a second-order sequential organization among recalled words, using the standardized formula derived by Tulving (1962). Results indicated: 1) A diversity in SO performance amongst the frontal lesion group depending upon specific lesion site. Subjects with dorsal frontal lesion scores were lower than the orbital frontal lesion group; and 2) Subjects with dorsolateral frontal lesions also obtained lower SO scores than non-frontal lesion subjects, but the SO of the orbital frontal and non-frontal lesion groups did not differ. We propose that the dorsal lateral frontal lobe plays an important role in the subjective organization of unrelated words in free recall, but the orbital sector does not.

507.9 CASE STUDY EVIDENCE FOR A CRITICAL AND SPECIFIC RIGHT OCCIPITAL-LORE CONTRIBUTION TO PERCEPTUAL IDENTIFICATION REPEITION PRIMING. D.A. Green*, I. *Sen, and J.J. McCarthy; Dept. of Psychology, Dep. of Radiology, Rush Medical College, Chicago, IL, 60612.
Two case studies are reported that suggest right occipital modulation of one form of visuospatial implicit memory but no critical role for that same brain region in conceptual implicit memory or in explicit memory. Subjects were 2 patients with unilateral left or right occipital resections. The performance of the left occipital patient was compared to 3 matched controls on a perceptual implicit test of word perceptual identification, a conceptual implicit test of word category exemplar generation (CE), and a conceptual implicit memory test of category exemplar generation (CE), with a matched word recall explicit memory test. For the PI and recognition study, subjects named aloud 24 words each presented twice. For the PI test, subjects identified list presented and masked words (24 repeated, 24 baseline). Perceptual priming was measured as the difference in identification response times between baseline and stimulus word presentation. For the CE test, subjects were given 12 category names and asked to provide 8 objects; 6 categories provided a baseline measure. Conceptual priming was measured as the number of correct responses produced for a list rated relative to baseline. For the CE test, category names were used as cues to recall stimulus word lists. The right occipital patient showed normal levels of perceptual priming, word category recognition, and cue recall accuracy, but PO priming. The left occipital patient showed normal magnitudes of priming and normal levels of recall and acquisition accuracy. These results (1) show a dissociation between the neural substrates supporting perceptual and conceptual implicit memory; (2) suggest that right occipital is a component of perceptual implicit memory; and (3) provide a reverse dissociation to that seen in global amnesia, with impaired implicit memory on the PI test despite intact explicit memory on the recognition test. Supported by McDonnell-Pew Cognitive Neuroscience Program.

507.10 VERBAL IMPLICIT RECALL OF SUBJECT PERFORMED TASKS BUT NOT VERBAL TASKS IN ALCOHOLIC KORSKOFF'S AMNESICS. T.W. PARKER. Department of Psychology, Augustana University, Camrose, Alberta, T4V 4A2.
Implicit recall of subject performed tasks (SPT's) versus verbal tasks (VT's) was tested for alcoholic Korskoff's patients with dementia amnesia and for normals. Tasks involved having subjects either perform (SPT) or describe (VT) an unnatural manipulation of pairs of common objects. Implicit recall tests, which made no mention of the earlier study phase, were then conducted after 10, 24 hours, and 30 days for each subject. No feedback was given during the tests. An overall ANOVA for repeated measures showed significant main effects for tests and for task type, and a significant test by task interaction. These results indicated that for both groups implicit recall of SPT's was markedly superior to that of VT's and that more decay over tests occurred for VT recall. This recall was more pronounced for Korskoff subjects. However overall Korskoff performance did not differ from normals, especially in recall of SPT's.
These results are seen as supporting the proposal that SPT's involve implicit memory components (Nilsson & Backmann, 1989) and hence are recalls contrastly to VT's that were mediated tasks. On the other hand, better recall of VT's by the normals suggests they may be using explicit memories to aid their recall.
507.11

Abnormal alcoholic amnesics (n=10), as defined by performance on a battery of verbal and visuospatial declarative memory tasks (e.g. memory for stories, memory for abstract designs), were also severely impaired in the Sternberg memory scanning paradigm compared to alcoholic non-amnesics (n=8) and healthy controls (n=9).

Standard tests of short term memory (e.g., digit span, block tapping span) yielded mixed results.

Procedural learning was intact in the amnesics. Learning to draw with only a mirror image as visual guidance improved normally within and between two consecutive daily sessions, with the amnesics eventually achieving performances identical to those of controls, despite the fact that they were initially much poorer.

On a new computerized version of the fragmented pictures task, administered over three consecutive days, the amnesics were unable to say (free recall) which famous faces they had seen the day before, but they were able to identify the masked pictures sooner (i.e., with a higher degree of masking) if they had seen them the day before than if the pictures were novel. Visual scanning of the facial stimuli, as assessed with an infrared eye-movement recording system (ASL EM-Motion 210) was not abnormal in either alcoholic group, providing yet another dissociation between procedural and declarative elements of task performance, and ruling out an attentional deficit as underlying impaired recall.

507.13
SLOW CORTICAL POTENTIALS IN CLASSICAL CONDITIONING. H. Flor, N. Birbaumer, W. Lutzenberger, and T. Elbert*. Inst. of Medical Psychology, Univ. of Tübingen, 7400 Tübingen, FRG.

The repeated occurrence of events with high probabilistic association elicits a distinct brain response, the contingent negative variation (CNV), which may be used as a tool in the description of the associative process during conditioning. It was assumed that the early component of the CNV (initial CNV, iCNV) might represent the amount of anticipatory arousal with respect to the US that is elicited by the CS and would thus be especially sensitive to changes in meaning of the CS over time. The late component of the CNV (terminal CNV, tCNV) should indicate preparation for the CR.

Experimental evidence from a differential conditioning study in humans indicates that the late CNV may be increased with varying emotional content as CS, electric shock as US, and autonomic and motor responses as UR/CR, indicated a clear lateralization of the iCNV on the side of the CR. A significant differentiation between the CS+ and CS− could be observed. The quality of conditioning was reflected in the height of the tCNV.

The data from this experiment suggest that the CNV may be regarded as a valid measure of the associative strength during classical conditioning.

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507.14
ELECTROPHYSIOLOGICAL INDICATORS OF READING DISABILITY OBTAINED IN THE FIRST AND THIRD GRADE. S.L. Miller*, Dept. of Psychology, University of North Carolina at Greensboro, Greensboro, NC 27412.

Fifteen reading disabled (RD) and fifteen non-reading disabled (NRD) subjects, selected as being "at-risk" for later reading problems, were compared on event-related potentials (ERPs) and behavioral measures. ERPs were recorded during a black-white discrimination task of visually presented letter and non-letter stimuli when the subjects were an average age of 6.7 years and again two years later. The RD, as compared to the NRD group, showed longitudinal stable reductions in neural activity (a) present within the initial 100-140 msec after stimulus presentation and (b) greater for letter than non-letter stimuli and larger over the left than right hemisphere at 180-240 (N2) and 460-600 (P3) msec after stimulus presentation. Behaviorally, individuals with a reading disability demonstrated slower and less accurate task performance. Both groups, however, did demonstrate a high level of task accuracy and showed faster performance during the black-white discrimination of letters as compared to non-letter patterns. These reductions in ERP amplitude are considered to reflect a reduction in selective neural processing for the reading disabled group during the task. The data suggest further that these deficits involve multiple levels of neural processing in the geniculate-striate visual processing system and are frequently greater over the left than right hemisphere. Supported by NIH Grant R03 NS0413-08.
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508.1 ANALYSIS OF SINGLE UNIT RECORDINGS FROM CEREBELLAR CORTEX OF CLASSICALLY CONDITIONED RABBITS. M.R. Fox*, D.J. Krupa, J.T. Tracy and R.F. Thompson.

1Dept. of Psychology, University of Southern California, Los Angeles, CA 90089 and Neurosciences Program, Univ. So. Calif., Los Angeles, CA 90089.

The cerebellum forms an essential portion of the neural circuitry necessary for the development and expression of the associative elements which mediate rabbit eyelid conditioning. An analysis of extracellular single unit activity recorded primarily from hemispherical lobule VI (HVI), but also from portions of anisiform cortex (Crus I and II) and the paramedian lobule (PM) of cerebellar cortex in classically conditioned rabbits was done to determine if there is an additional source of NOS activity in hippocampus, or that effect of NOS inhibition on either control responses or the enhancement of the hippocampal LTE involves a persistent modification in some properties of postsynaptic AMPA receptors. [Supported by AG09219 and ONR].

508.2 ASSOCIATIVE LONG-TERM DEPRESSION REVEALED BY FIELD POTENTIAL RECORDING IN RAT CEREBELLAR SLICE. C. Chen* and R. F. Thompson, Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Field potential recording in the cerebellum is essential for understanding long-term plasticity because it reflects population changes and is stable. The difficulty in obtaining well-defined field potentials in cerebellar slice has been a major obstacle in studying long-term depression (LTD) (Ito, Ann. Rev. Neurosci., 1989). We have reliably recorded the field potential of Purkinje cell (PC) dendrites evoked by parallel fiber (PF) stimulation in young adult rat cerebellar slice (sagittal section of 400 um), and have identified TTX-sensitive action potentials and AMPA receptor-mediated EPSP (elicited by 10 μm CNQX). The response evoked by activating the climbing fiber (CF) through the white matter stimulation can also be blocked by CNQX. By using the field potential recording in the absence of GABA blockers, consistent LTD (30% decrease from baseline EPSP amplitude, 60 min post pairing, n=9) of PF-PC synapses can be induced by 600 pairings (1 Hz) of a PF stimulus (0.1 ms) followed 250 ms later by a CF stimulus. The PF-EPSP in the nonpaired pathway recorded 300 um away from the paired one shows a small increase (18%+15%, n=9), indicating that the LTD is localized in the paired pathway and the slice is healthy over the course of study. This new interanimal protocol for LTD induction may have some relevance to behavioral motor learning.

508.3 CHANGES IN THE BINDING PROPERTIES OF GLUTAMATE RECEPTORS FOLLOWING LONG-TERM ENHANCEMENT (LTE) OF PERFORANT PATH SYNAPTIC TRANSMISSION IN AWAKE RATS. G. Ra
to*, G. Tocco, M. Sandr
d, R. F. Thompson, B. L. McNaughton, and C. A. Barnes*. Neurosciences Program, Univ. So. Calif., Los Angeles, CA 90089, and 1RAL Div. Neuro
systems, Memory and Aging, Univ. Arizona, Tucson, AZ 85714.

The mechanism of long-term synaptic enhancement (LTE) is still vigorously debated, and there is evidence for both pre- and postsynaptic changes. We have examined the binding properties of glutamate receptors following induction of LTE at perforant path-granule cell synapses of awake rats. Anesthetized adult male Fisher-344 rats were implanted bilaterally with a monopolar stimulating electrode in the perforant path and a recording electrode in the hilus of the dentate gyrus. Following recovery, 8 rats received 11 high-frequency perforant path stimulation sessions (10 25 msc 400 Hz bursts at 10 sec per session). Prior to the stimulation, and 10 high-frequency sessions over 6 days. Equal an amount of stimuli were delivered to the opposite hemisphere at low frequency (1 Hz) to yield an internal control. Either 4 hours (8 rats) or 24 hours (6 rats) following the final stimulation session, rats were sacrificed and their brains rapidly dissected and frozen. Quantitative autoradiography of [3H]-AMPA and [3H]-TCP binding to frozen brain sections was used to examine the AMPA and NMDA subclasses of glutamate receptors, respectively. LTE induction resulted in a significant increase in AMPA binding in the dentate gyrus (stratum moleculare) as compared to the hemisphere receiving low-frequency stimulation. No significant changes in [3H]-TCP binding were observed in any treatment group. These results suggest that the expression of hippocampal LTE involves a persistent modification in some properties of postsynaptic AMPA receptors. [Supported by AG05142 and the McKnight Foundation to RFT, NSF to MB, AG03376 to CAB, and ONR to BLM].

508.4 SYNAPTIC ACTIVATION OF TRANSCRIPTION FACTORS: DISSOCIATION OF STIMULUS REPETITION AND LTE IN HIPPOCAMPAL SYNAPTIC TRANSMISSION. T.E. Schapf*, M. Baraban*, G.D. Stevenson*, B.L. McNaughton, G. Rao and C.A. Barnes. 1Dept. Neuroscience, Johns Hopkins University, Baltimore, MD 21205, and 2ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

High-frequency synaptic activation of hippocampal granule cells induces a rapid increase in mRNA of specific transcription factors, suggesting that this activity is involved in long-term synaptic enhancement (LTE or LTD; Cole et al., Nature 404, 474, 1990). This genomic response appears to include c-fos, jun-B, and jun-D; however, the reproducibility and magnitude of these responses has been variable and may be altered by anesthesia, injury and the stimulation protocol. To evaluate the relationship between LTE and genomic responses further, implanted, unanesthetized rats were given a receiving a stimulation protocol sufficient to induce LTE lasting about 3 days (10, 25 msc trains at 400 Hz, separated by 10 sec). In each of 18 preparations, n=68 mRNA was induced to levels identical to those induced by maximal electroconvulsive seizures (MCS) 30 min after the stimulus. By contrast c-fos and c-jun were not detectably increased in any of the high frequency preparations but were strongly induced by MCS. Jun-B mRNA was weakly induced, relative to MCS in all preparations. With more repetitions of high frequency stimulation at a shorter interval (i.e., 50, 20 msec trains at 400 Hz, separated by 20 sec), there was no further LTE induction; however a weak c-fos mRNA response was observed. Our results indicate a differential threshold for synaptic activation of these transcription factors and suggest a special role for c-fos in LTE. [Supported by AG0219 and ONR].

508.5 NITRIC OXIDE SYNTHASE INHIBITION IN VIVO HAS NO EFFECT ON HIPPOCAMPAL SYNAPTIC ENHANCEMENT OR SPATIAL MEMORY C.A. Barnes*, B.L. McNaughton*, D.S. Redel, C.D. Ferris, and S.H. Snyder.


In vitro studies suggest that nitric oxide translates induction events into expression of long term enhancement (LTE/LTP) of hippocampal synapses (Boehme et al., 1991, Shoman & Madison, 1991). However, NO synthesis is induced post-synthetically by a NMDA receptor dependent mechanism, but appears to increase transmitter release from terminals near the site of NO release; however, such a presynaptic mechanism for LTE has been the subject controversy (Malmow & Tsien, 1990; Bokkers and Stevens, 1990; Foster and McNaughton, 1991; Menade and Nicoll, 1992). We have studied the induction of in vivo treatments of nitric oxide synthase inhibitor (NOARG), an irreversibly inhibitory NO inhibitor of NOS (Dwyer et al., 1991). Rats were implanted bilaterally with electrodes for stimulation and recording of perforant path synaptic potentials in fascia dentata. Response was counted daily on a 100 μA p. j. of NOARG (20-50 mg/kg) or vehicle (n=6 rats) were begun. On days 10 and 11, spatial memory was tested using the Morris water task. On day 12 and 13, unilateral NOARG (20 mg/kg) was given twice at a 2 h interval. Fractional enhancement of the EPSP and population spike 24 hours after HFS was compared with the control hemispheres between groups. Enzymatic assay indicated at least 90% inhibition of NOS. There was no effect of NO inhibition on either control responses or the enhancement of the EPSP or population spike, and no effect on spatial learning. We conclude that either there is an additional source of NO activity in hippocampus, or that the form of LTE blocked in vitro in CA1 by NOS inhibition does not reflect the same process as is induced in vivo in PD. [Supported by NIMH].

508.6 LACK OF AN EFFECT OF TEMPERATURE ON TETANUS INDUCED ENHANCEMENT OF SYNAPTIC FIELD POTENTIALS IN CA1. L. Shen*, C. W. Gao, C. A. Barnes and B. L. McNaughton, ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Previous studies (Foster & McNaughton, 1993) suggest that low temperature (22°C) results in a substantial increase (compared to 32°C) in the magnitude of synaptic enhancement induced by pairing 2 Hz stimulation of single or small numbers of presumed parallel fiber axons intracortically with depolarizing current pulses. The excess EPSP enhancement was associated with an apparent increase in quanta content that was not seen at 32°C, where only changes in quantal size were observed. Curiously, although quantal size changes were apparently blocked by APV or the omission of the depolarizing pulses during 2 Hz stimulation, the apparent increase in quantal content was not. Because reduced temperature in some systems leads to an increase in the initial elevation of the field EPSP (PTP), but there was little effect on its apparent decay rate, and no effect on the magnitude of the persistent phase of enhancement (about 40%). Apart from the possibility that the previous results reflect statistical error (i.e., false positive results), we see two possible explanations. There could be important differences between pairing induced synaptic enhancement vs. the synchronous high frequency stimulation. Alternatively, it is quite possible that there are persistent, temperature dependent effects of repetitive stimulation on axonal excitability. If there were fewer conduction failures following 2 Hz stimulation at 22°C, this would appear as an apparent increase in quanta content. These possibilities are under investigation. [Supported by ONR].

We think of experiences as samples drawn from a probabilistic environment, and suggest that the neocortex adjusts its synaptic strengths to maximize its ability to respond appropriately to future important events drawn from the same environment. Finding satisfactory connection strengths requires gradual adjustment to allow the overall direction of weight changes to be guided by an adequate balance between short-term and long-term memory. In simulations, such gradual changes lead to powerful cognitive representations, but a key requirement is that new information must be accommodated slowly. Weight changes large enough to store arbitrary responses are generated by the cortex and if this information recurs in the midst of other experience, it gradually becomes integrated into the cortex. One way the information may recur is via recall from hippocampus; in such cases hippocampal activity acts as a teacher to the cortex. Cortical learning must be gradual; memories initially dependent on hippocampus can only gradually lose this dependence. Thus, graded retrograde amnesia is seen as a reflection of a key design characteristic of the two-part memory system. A further consequence is that the connections in the pathways between the cortex and the hippocampus must themselves be stable. This notion has led us to consider a new possible function for area CA4 L for its relatively point-to-point reciprocal connections (Tamamaki pers. com.) with entorhinal cortex, for the low density of NMDA receptors found in stratum lacunosum, and for the ability of direct EC inputs to drive CA1 pyramidal cells (Ykecl & Berger, 1991). (Supported by grants M147566 & MH30835 to JLM and M146623 to BLM)

QUANTIFICATION OF WHAT IT IS THAT HIPPOCAMPSAL CELL FIRING ENCODES. W.E. Skaggs*, B.L. McNaughton, ARL Div. Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

We describe a information theoretic approach to determining what it is that neural firing encodes, obtaining a measure of the amount of information (bits) that each spike conveys about any particular measured variable(s). The technique treats a neural cell as a communication channel whose output is the variable(s) and whose output is the spike train. Using data recorded from hippocampal cells in freely moving rats, our information measure is compared to several other possible measures of possible-depency of activity, including entropy (the mean square firing rate divided by the square of the mean), coherence (the correlation of firing rate with the firing rate at neighboring locations), dispersion (the RMS distance from the center of the firing rate distribution, weighted by firing rate), and existence of a place field, determined using a method described by Muller et al. (1987). As a control, each measure is computed 100 times with time-shifted relative to the animal's position and behavior data, and a Z-score is obtained from which the significance can be determined. The information measure is the most sensitive detector of place-dependency, with sparsity following closely. Dispersion is only useful when the cell has a strong single place field. Coherence is less sensitive and depends on selecting good thresholds for firing rate and field size. We discuss several issues, including: how to bin data to obtain the most meaningful measures; what to use as the prior probability distribution for the information measure; and the critical importance of an appropriate control. Finally, we use the information measures to quantitate the contribution of place, head direction, and velocity to a cell's activity, and compare the results with the outcome of a multiple regression analysis. (Supported by MH146623)

SPATIAL FIRING CHARACTERISTICS OF DENTATE GRUSSY GRANULE CELLS. M.W. Jung*, B.L. McNaughton and T.W. Abel. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Theoretical arguments concerning the role of the dentate gyrus in pattern separation and associative memory (Marr, 1971; McNaughton and Nadel, 1990) suggest that granule cells should exhibit sparse coding, i.e., they should fire only rarely at high rates, and should have tight 'place fields. In contrast, several previous attempts to characterize granule cells in vivo have concluded that they fire at high rates, with no obvious place fields and are similar to 'beta' cell interneurons found throughout the hippocampus; however, Mizumori et al. (1989) found, in anaesthetized animals, that the high and low rate cells in the granular layer could be separated on the basis of the failure of high rate cells to be inhibited during paired-pulse inhibition of the population spike, and the ability to drive high rate cells at low rates (abnormal population spike responses in the granular layer). They concluded that granule cells fire at low rates. We have studied the spatial and temporal firing properties of cells located (on the basis of histology and spatial projection in and near the granular layer) in or near the granular layer of the dentate gyrus. Using stereotrode recording and real-time cluster plotting of spike parameters that facilitates the detection of very low rate cells, we find that most (38/44) cells in the layer have low rates (0.01 - 0.5 Hz), cannot be driven orthogradically below population spike threshold, and exhibit spatial selectivity equal to or better than the average CA3 pyramidal cell.

Firing fields are highly directional. A number of these cells exhibit unusual spatial firing distributions consisting of three or four very tight fields distributed over the radial maze surface. Although the list of the identity of low and high rate cells is by no means definitively resolved, the weight of evidence currently supports the conclusion that granule cells exhibit temporally and spatially sparse encoding. (Supported by NS20331)


A microdrive array capable of independent vertical adjustment of 12 recording electrodes ('tetrodes') each containing 4 recording sites and separate inputs fed into simultaneously isolate multiple single unit activity from the somatosensory regions of parietal cortex and hippocampal formation of the freely moving rat. Spike and EEG data were preprocessed using a custom built, computer controlled, 56 channel amplifier/filter array, and collected using seven synchronized 80466 computers. Animals were trained to perform a forced-choice alternation task on a two-armed maze for food reward. Animal position and head direction were recorded by direction were recorded by video camera while single unit activity was monitored. Activity has been successfully recorded from both cortical and hippocampal regions; however, only the cortical data have been analyzed to date. During a single recording session, 61 cells in sensory-motor cortex were simultaneously monitored while the animals either performed the task or were subjected to somatosensory stimulation. Of these cells, 30% showed various degrees of turn biased firing, 20% had preferred responses during linear running behavior, and 50% had non-specific or non-motion related responses. A preliminary analysis of the ensemble activity in the population of cells showed that the population vectors composed of firing rates for each cell over 100 msec to 1 sec time bins. The correlations between vectors at different times over the course of a single maze trial were compared and repeating vector patterns which encoded turning direction were identified. Ongoing work involves extending the analyses of ensemble encoding of behavioral state and spatial information. (Supported by NSF, NS20331 and MH146623)

PLACE FIELD SPECIFICITY DEPENDS ON PROXIMITY OF VISUAL CUES. K.M. Gothard*, W.E. Skaggs, B.L. McNaughton, C.A. Barnes and S.P. Youngs. ARL Division of Neural Systems, Memory, and Aging, University of Arizona, Tucson, AZ 85724.

Gaese et al. (1991) showed that the "place fields" of hippocampal complex spike cells are more specific when visual cues are proximally situated. Using a quantitative measure of place field specificity, we see that the place cell's specificity is determined by the relative proximity of an object in an opaque cylinder with cue cards on the walls of the cylinder (proximal cues), and in a transparent Plexiglas cylinder with a visually similar array of cues on the walls of the transparent cylinder. The cells of the two environments were alternated. Between trials, the animals were allowed to experience the whole room. Using either multiple regression or factorial ANOVA to account for contextual changes in mean running speed, mean firing rate, trial order, and environment, we found that place cells (89) showed significantly (p < .005) higher specificity in the opaque cylinder. We also found that the overall distribution of place cell mean firing rates is 25% more sparse in the transparent cylinder. These results suggest that the distal-cue environment is represented by a smaller population of cells with less specific place fields. This outcome supports the theory that place cell activity is partially determined by the "local view" seen by the animal, because in the distal-cue environment there is less parallax of visual cues as the animal moves from place to place. Order also had a significant (p < .01) effect on specificity, with place fields more specific in the second of the two consecutive sessions. As the second session was always preceded by exploration of the entire room, this may reflect the short-term effect of the intertrial experience. (Supported by NS20331)

DECREASE IN THE INFORMATION CONTENT OF HIPPOCAMPSAL CA1 CELL SPATIAL FIRING PATTERNS IN THE DARK. E. Markus*, C.A. Barnes, B.L. McNaughton, V. Glaedt, T.W. Abel and P.A. Gloor. ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

In adult rats some hippocampal cells exhibit similar "place fields" in a given environment both under light and dark conditions as long as the light condition precedes the dark (McNaughton et al., 1989; Quirk et al., 1990). We were interested in quantifying the relationships between spatial firing in the light and dark and comparing young and old rats.

Young adults (9 month) and old (22 month) F-344 rats were trained to perform a forced choice task on an open arm radial maze in a room. The rat was placed on the center of the maze in an illuminated room and subsequently ran alternate trials with the room either illuminated or darkened. Cells were recorded in the CA1 pyramidal cell layer using stereo electrode recording methods.

Place fields were less specific and less reliable in the old rats and both age groups exhibited reduced place field specificity in the dark. There was no significant interaction between age and light condition. (Supported by AG03376 and NS20331).

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M. Ceccarelli, D.L. Korel, C.A. Barnes and R.L. McNaughton.

Exploratory behavior results in increased perforant path evoked EPSPs and decreased population spike area and onset latency. These changes, collectively called short-term exploratory modulation (STEM), can be predicted from the amount of exploratory behavior (Green et al., 1990; Sharp et al., 1989). It is unknown whether STEM reflects information storage per se or some less specific process, such as the magnitude of STEM with the amount of exploration during recording sessions in a cue-filled environment and with spatial learning ability in the Morris water task in young and old Fisher-344 rats. There was a significant relationship between the amount of STEM and spatial learning ability. EPSR growth was significantly correlated with spatial learning, measured by latency to escape to a hidden platform (r = 0.308, p = 0.052, and percent time spent in the target quadrant during a free-swim probe trial (r² = 0.434, p > 0.011). There was also a correlation between exploration and probe trial quadrant search (r = 0.362, p = 0.003). When changes in the EPSR (STEM) age, and exploratory behavior were entered into a stepwise regression analysis, synaptic change (STEM) emerged as the best predictor of the variability in spatial learning. The relative spike attenuation in a recording session was significantly correlated with the amount of exploratory behavior (r² = 0.170, p = 0.025, but not to spatial ability. These data provide support for the hypothesis that STEM may be involved in spatial memory. It remains to be determined, however, whether this relationship reflects the actual storage of spatial information via specific synaptic alteration, or whether STEM reflects an important, but non-specific modulator of information transmission through the hippocampus. [Supported by AG03756 and ONR].

508.15


Electricaly induced hippocampal synaptic enhancement (LTP/LETP) is believed to reflect activation of a physiological memory mechanism. One of the cornerstones of this theory relies upon the observation that experimental saturation of LTP/LTP, a substantial fraction of perforant path terminals leads to an impairment in the acquisition of different spatial memory tasks. Two of these tasks include the Barnes circular platform task, in which animals seek to escape from a brightly illuminated uniform surface to a dark tunnel located under one of 18 separarly located holes (McNaughton et al., J. Neurosci., 1986), and the Morris water task, in which rats are required to learn the location of an escape platform located just below the surface of a pool of water (Castro et al., Nature, 1990). Recently, several groups of investigators have attempted, without success, to replicate the results of Castro et al. (Morris et al., pers. comm.; Cain et al., pers. comm.; Sutherland et al., pers. comm.) in the belief that these failures might reflect strain differences in the animal subjects used. We have attempted to replicate the Castro et al. study using both (albino) F344 and hooded Long-Evans rats. We found no effects of LTP on probe-trial quadrant search time, regardless of whether the animals were tested immediately (14 rats total) or 24 hours (12 rats total) following the last stimulation session. There were also no strain differences. At present, we have no good explanation for the discrepancies, apart from the smaller number of rats used by Castro et al.; however, there are several variables under investigation including pool size and previous experimental history of the animals. Any further results will be reported. [Supported by AG03756, AG05401, and ONR].

508.16


Short-term exploratory modulation (STEM) is a form of naturally occurring synaptic plasticity that has been observed in the rat dentate gyrus (Sharp et al., 1989). It occurs during exploration, learning of a task or simply if the rat is placed in a novel environment. STEM shares some, but not all properties of LTP induced by high frequency stimulation (Eriksson et al., 1991; McNaughton et al., 1991). Typically, when using only low frequency stimulation of the perforant path, the population EPSS increases and the population spike (FS) decreases during exploration. These effects persist for many minutes. Noradrenaline (NE) has been shown to modulate the induction of LTP (Bliss et al., 1983) and also produces lasting effects of its own (LaCalie & Harley, 1985; Stanton & Survev, 1985). We tested the effect of STEM, a neurotoxin that depletes forebrain NE, on the generation of STEM. Rats with chronically implanted electrodes (N=10) were trained to run a forced choice (FC) task on the radial arm maze. The perforant path evoked response was measured before and after behavioural testing to assess the amount of STEM generated, both before and after 50PI4 treatment. This treatment had no overt effect on the behavior or the baseline EPS and FS. It did, however, reduce the magnitude of the increase in the EPSP by approximately 50% and the decrease in the FS by approximately 50%. Systemic administration of either of the adrenergic antagonists resulted in dose-dependent reductions in STEM. These results indicate that NE is necessary for the full expression of STEM; however, whether NE produces STEM directly or whether it modulates the neuronal activity required for STEM is not as yet known. [Supported by ONR, NSF & McDonnell-Pew Fdn.]

508.17


During free exploration, the field synaptic potential (epp) in the rat dentate gyrus gradually increases whereas the spike amplitude and latency is reduced (Sharp et al.,1989, Psychobiol., 17, 257-69; Green et al.,1990, J. Neurosci., 10, 1455-71). These effects (STEM) reflect the exploration and have been interpreted as altered synaptic efficacy associated with temporary memory for recent events. We asked whether STEM is related to increased brain temperature, which gives similar changes of potentials. Rats were chronically implanted with a stimulation electrode in the perforant path, a recording electrode in the dentate granular layer and a thermistor at the same depth in the contralateral hemisphere. Field potentials, brain temperature and exploratory activity were recorded simultaneously. Exploration in a novel environment produced both STEM and enhanced brain temperature (‡ 2.5°C) which increased and decayed with identical time courses. In spite of vigorous exploratory activity, STEM was prevented by keeping the rat at a constant brain temperature using radiant heating. This temperature was equal to the maximal value observed in the same rat during control exploration. In other experiments, the changes in field potentials were either larger or the lower the brain temperature at the start of exploration. Walking on a motorized treadmill produced both STEM and increased temperature.

Taken together, these results imply that STEM is due to increased brain temperature and also question any relation to memory processes.
509.1
A Ca2+-DEPENDENT PROCESS MEDIATES 15,3R-ACPD-INDUCED POTENTIATION OF NMDA RESPONSES IN RAT HIPPOCAMPUS: J. Harvey and C.L. Collingridge, (SPQR: Brain Research Association), Dept. of Pharmacology, The Medical School, Univ. of Birmingham, Birmingham, UK. We have previously reported that 15,3R-ACPD selectively potentiates responses to NMDA but not AMPA in hippocampal slices (Harvey et al 1991, Br. J. Pharmacol. 104, c79) and these findings have been confirmed in single cells by Antiketis et al. (Eur. J. Pharmacol. 1991, 205, 327). We have gone on to investigate the mechanisms of this potentiation, using a reverse gap method for hippocampal slices. Exposure of slices obtained from female rats (5-8 weeks old) and perfused with a standard Mg2+-containing solution at 28 °C. Saroprostamine (1 μM), a PKC inhibitor (chelerythrin in 10 μM, which depletes intracellular Ca2+ stores, and bromophenacyl bromide (50 μM), a PLA2 inhibitor, did not affect this potentiation. However these compounds were active as they prevented full expression of long-term potentiation, as reported previously (Matthews et al. 1991, Neurosci., Latt. 121, 259; Harvey et al. 1992, Neurosci., Latt. in press; Massicotte et al 1990, Brain Res. 537, 49). In a further 5 slices perfused with Ca2+-free medium there was no effect of this 15,3R-ACPD-induced potentiation of responses to NMDA. These findings suggest, therefore, the involvement of a Ca2+-dependent process in this potentiation, although the actual mediator has yet to be identified.

509.3
QUANTITATIVE AUTOGRAPHY OF ENHANCED [3H]MK-801 BINDING IN TRAEC CONDITIONED HIPPOCAMPUS: L.T. Thompson, M. Dobrovich, & J.E. Dimonoh, Dept. of CMS Biology & of Pharmacology, Northwestern University Medical School, Chicago, IL 60611. NMDA receptors may be critical in the neuronal plasticity underlying associative learning, and are abundant in dentate fields of hippocampal CA1 and dentate neurons. In rabbits, agonists of the glycine site on the NMDA receptor facilitated acquisition of hippocampal-dependent but not non-competitive antagonist blocked acquisition (Disterhoth et al., 1990; Thompson et al., 1991). Preliminary work indicated delay conditioning enhanced [3H]MK-801 binding in whole hippocampal homogenates (Thompson et al., 1990). The present study used quantative autoradiography to determine if learning-dependent changes in MK-801 binding are localized to discrete hippocampal neuronal populations after hippocampal-dependent 500 micr trace conditioning. Brain sections (20μm) from trained, pseudotrained, or naive rabbits were labeled with [3H]MK-801 in 30nM HEPES buffer (pH 7.4) containing 100 μM glutamate, 100 μM glycine, and 1 mM EDTA for 2.5 hr at 23°C (Subramanian & McGoun, 1990). Non-specific binding was determined in the presence of 200 μM ketamine. Autoradiographs were quantitated with a high resolution Macintosh® (imagining system and NIHIM software (image 1.44). Hippocampal [3H]MK-801 binding (20X) exhibited no denervation or left-right gradient in controls. Trace conditioning significantly enhanced whole hippocampal [3H]MK-801 specific binding in saturation studies (1-40 nM). Small enhancements were seen in the dentate gyms, but much larger increases in binding were observed in CA1. This effect was lateralized, with somewhat greater increase in binding seen in the CA1 region contralateral to the conditioned eye. These results suggest that alterations in NMDA receptor number (or in functional receptor number) may underlie some of the CA1-specific learning-dependent changes reported earlier (De Jonge et al.).

509.5
NMDA ANTAGONIST MK-801 IMPAIRS ACQUISITION OF LATENT INHIBITION FOR A PREEXPOSED FLAVOR STIMULUS. E. Billette, T.L. Linkhart and M.L. Murphy, Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC 27599. Previous studies have shown that simple exposure to a stimulus will retard later conditioning of that stimulus (latent inhibition), and that septohippocampal circuits play a critical role in mediating the effect. The present study investigated whether the NMDA antagonist MK-801, which blocks induction of LTP in the hippocampus, would also impair acquisition of latent inhibition. Rats were initially adapted to restricted water access (20 min/day). Separate groups of saline or MK-801 (10.08 mg/kg, sc) 35 min prior to exposure to a 2%/w/w saccharin solution or to tap water. A fifth group of rats received MK-801 immediately after preexposure to saccharin. Two days later all groups received a conditioning trial in which the saccharin was paired with delayed illness (0.3M LiCl, 63.5 mg/kg, ip). Subsequent tests showed that rats preexposed to saccharin after receiving saline injections acquired significantly weaker aversions than did non-preexposed rats (latent inhibition). Rats that received MK-801 before or immediately after saccharin preexposure, however, displayed saccharin aversions like those seen in non-preexposed rats (no latent inhibition). Various aspects of the data suggest that MK-801 disrupted latent inhibition because of its effects on learning, and not because of other possible effects of the drug (e.g., sensorimotor deficits). These results therefore support the idea that NMDA receptors in limbic system circuits play a role in certain forms of learning and memory.

509.9
NMDA ANTAGONIST MK-801 PRODUCES A DOSE-DEPENDENT IMPAIRMENT ON SPATIAL REVERSAL LEARNING IN THE RADIAL ARM MAZE. L.H. White*, K.R. Austin, and M.L. Shapiro, Dept. of Psychology, McGill Univ., Montreal, Quebec, Canada, H3A 1B1. Many experiments have shown that MK-801 blocks or retards spatial learning in an unfamilial environment. Several studies have investigated the effects of MK-801 on learning a new subset of spatial relationships in a familiar environment. Rats were trained on a version of the radial arm maze task which requires the rats to enter to obtain four of eight arms to retrieve a food reward (0.48 RAM). Upon reaching criterion performance, they were assigned to one control, and either 62.5 mg/kg, 100 mg/kg, or 125 mg/kg of MK-801 (n9 per group). Injections were given 1P 30 minutes prior to training. Performance was assessed for 3 days. MK-801 had no effect on working and reference memory (WM and RM). Each rat was then tested on a RM reversal task by baiting the set of arms opposite to those used for the initial acquisition. More errors were found between the groups on blocked trials of RM or WM errors throughout training. However, the 125 mg/kg MK-801 group took significantly longer than the other groups to learn the RM reversal task compared to acquisition (trials to criterion reversal minus trial to criterion acquisition). The 62.5 mg/kg and 100 mg/kg groups did not differ significantly on this difference score. The delayed acquisition of the RM reversal task for the 125 mg/kg MK-801 group is consistent with one interpretation that MK-801 impairs learning a subset of spatial relationships in a familiar room by inhibiting the ability to suppress previously learned information.

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THURSDAY AM
MK-801 IMPAIRS MEMORY FORMATION IN THE 2-DAY-OLD CHICK
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The amnesic effect of MK-801, a NMDA receptor antagonist, was demonstrated in chicks trained on a 1-trial passive avoidance task. Groups of chicks were given bilateral injections into the intermediate medial hyperstriatum ventrale 45, 30, or 15 min pretraining, using saline or 0.0015, 0.015, 0.15, 1.5 or 15 nM MK-801. In tests at 24 h, 1.5 nM and 0.15 nM MK-801 produced significant amnesia when injected 15 min or 30 min pretraining when compared to saline controls (p<0.01). No other dose of MK-801 produced amnesia. These results indicate that NMDA receptor activation is important for learning and memory formation in the chick. The appearance of amnesia after injections at 15 min or 30 min pretraining is consistent with the findings of other researchers, in that MK-801 has been shown to have maximal effect at about 30 min. Further experiments are necessary to determine if activation of NMDA receptors is important for the acquisition or retrieval of a task.

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509.13
EFFECTS OF PHENCYCLIDINE AND NALOXONE ON LEARNING OF A SPATIAL NAVIGATION TASK: AND PERFORMANCE OF A SPATIAL DELAYED NON-MATCHING TO SAMPLE TASK. M. Dakis, J. S. Martinez, R. P. Kaspar, and P. Jackson-Smith, Dept. of Psychology, Univ. of Utah, Salt Lake City, UT 84112
Rats were trained on a dry-lard version of a spatial navigation task (cheese board) under the influence of i.l. injection of 10 mg of Phencyclidine (PCP, an antagonist of the NMDA receptors), 13 mg of naloxone (an antagonist of the opiate receptors), or saline, directly into the dorsal hippocampus. Rats received 2 blocks of 4 trials with different starting points, relative to the goal (food) per day, for three consecutive days. Based on a distance traveled measure, results indicated that relative to saline controls, the PCP group learned normally within a day, but displayed forgetting between days. Conversely, the naloxone group displayed disruption of learning within a day, but displayed normal learning between days. These preliminary results indicate that the NMDA receptors mediate consolidation of spatial information, the opiate receptors mediate short-term memory representations, and that the processes can operate independent of each other.

This conclusion is further supported by results obtained from rats previously trained on an 8-arm spatial delayed non-matching to sample task with delays of 1 and 30 minutes. The rats were treated exactly the same as in the previous experiment. In this case, PCP had no effect, however naloxone disrupted performance on the 30, but not the 1 minute delay. Because it is assumed that the latter task measures the operation of short-term memory representations, but not consolidation of spatial information, the results lend additional support to the hypothesis that opiate receptors mediate short-term memory and NMDA receptors mediate consolidation of spatial information.

509.15
A NITRIC OXIDE SYNTHASE INHIBITOR RETARDS ACQUISITION OF THE CLASSICALLY CONDITIONED RABBIT EYEJED RESPONSE. M. Todd Allen and J. E. Steinmetz, Program in Neural Science, Psychology Dept. Indiana University, Bloomington, IN 47405.
Nitric oxide (NO) may be involved in long term depression (LTD) processes in the cerebellum. In this present study, we tested the effects of L-nitro-arginine methyl-ester (L-NNAME), a NO synthase inhibitor on classical conditioning, a learning procedure thought to involve the cerebellum. Rats were given L-NNAME at 3 doses (10, 25, 75 mg/kg) or the stereoisomer D-NNAME (25 mg/kg) while being trained in the classically conditioned rabbit eyelid response paradigm (CS-none, US-airpuff). The L-NNAME groups had significantly lower percentages of conditioned responses than did the D-NNAME controls over the first several days of training. No effect of L-NNAME was obtained in rabbits that had been previously conditioned to criterion after injection of the control substance, D-NNAME. Preliminary results from conditioning with L-NNAME while recording multiple unit activity in the interpositus nucleus indicate that the training-related neuronal model characteristic of the interpositus nucleus is delayed similarly to the appearance of the conditioned response. These data suggest that blocking NO synthesis may disrupt cellular processes normally involved in the acquisition of classically conditioned responses.

509.14
N-methyl-D-aspartate (NMDA) receptor activation has been linked to enzymatic production of nitric oxide (NO) by nitric oxide synthase (NOS) (Garthwaite, 7496, 145, 1991). NMDA receptor antagonism retards formation of long-term potentiation (LTP) and impairs acquisition in maze tasks (Izquierdo, TIPS, 12:128, 1991). These observations have been linked to intracellular events involving NO production as a retrograde messenger increasing presynaptic glutamate release. Specifically, when NOS is inhibited, LTP is blocked in vitro (Schuman & Madison, Science, 254:1503, 1991). We examined whether NOS inhibition impairs learning of male F-344 rats (9 mo) in a 14-unit T-maze. Previous results in this maze indicated that NMDA receptor channel antagonism impairs acquisition but not retention (Scangler et al., Pharm. Biochem. Behav. 40:949, 1991). In the present study, rats were pretrained in 1-way active avoidance to a criterion (13/15 avoidance) in a straight runway. The next day, rats received i.p. injections of 0.9% NaCl as controls or N'-nitro-L-arginine (N-Arg) to block NOS (5.0, 4.5 or 6.0 mg/kg) 30 min before maze training. During 15 trials, rats were required to negotiate each of 5 segments within 10 s to avoid footshock (0.8 mA). Performance variables included errors (deviations from correct pathway), return time from start to goal, shock episodes and duration. N-Arg treatment impaired performance in all variables in a dose-dependent manner. Controls and rats treated with 3 mg/kg N-Arg were retested in the maze 7-10 days following training, with half being injected with N-Arg (6 mg/kg) 30 min in advance. Performance under these conditions was affected minimally indicating that NOS inhibition primarily impairs acquisition.

BIOLOGICAL RHYTHMS AND SLEEP V

510.1
INHIBITORY RESPONSES TO PRESSURE EJECTED NEUROPEPTIDE Y IN RAT SUPRACHIASMATIC NUCLEUS NEURONES IN VITRO. F.M. Sidney and B. J. Jones Smith.Kline Beecham Pharmaceuticals. Coldharbour Road, The Pinnacle, Harlow, Essex, UK, CM19 5AP. SPON: Brain Research Association
Neuropeptide Y (NPY) has been shown exclusively to increase the spontaneous firing rate of SCN (Suprachiasmatic Nucleus) neurons in hamster hypothalamic slices in vitro when administered by pressure ejection (1), but predominantly to inhibit the firing of both rat (2) and hamster (3) SCN neurons when perfused in the bathing fluid. Using hypotonic slices prepared from rats, and standard extracellular recording techniques we found that pressure ejection of NPY (50-200mM, 10 psi, 0.1 to 3 seconds) inhibited the spontaneous firing rate of 75% (51/68) of SCN neurons tested. This refutes the suggestion that the application explained the discrepancies between rat and hamster. No stimulatory responses were seen, the remainder of the cells being unaffected by NPY.
Neurons could be distinguished by the depth and duration of the response to NPY, and by a monophasic or biphasic return to previous firing rate. In a second series of experiments designed to investigate NPY responses in the SCN at various circumadian times, we found no difference in the frequency of inhibitory responses (to 50mM NPY) during the light phase (8%) and the dark phase (81%). However, whereas the durations of responses during the light phase were equally distributed, the duration of responses during the dark phase were predominantly long (20 to 45 min).
(2) Albers et al. (1990) Am. J. Physiol. 258 R376-R382

510.2
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The suprachiasmatic nucleus (SCN) receives direct visual afferent projections from the retina via the retinohypothalamic tract (RHT), as well as from the lateral geniculate nucleus via the tracts of the hypothalamic nucleus (TGN). Neuropeptide Y-containing fibers originating from the GHT appear to modulate circadian rhythmicity. Therefore we chose to investigate the relationship between acetylcholine and NPY receptor binding to the SCN in the golden hamster. The RHT was traced by implantation of carboxyane dye Dil into the distal end of one optic nerve (n=7). NPY receptors were localized using receptor autoradiography (n=11). Sections were labeled with [125]p peptide YY +/- unlabeled peptide YY. NPY receptor distribution was seen primarily in the ventral SCN in the central portion of the nucleus, but shifted laterally in more caudal sections. This pattern closely corresponded to RHT projections from the retina. These findings provide additional evidence that NPY is involved in a circadian regulation within the SCN, and suggest that both the RHT and GHT project to the ventrolateral portion of the SCN. Supported by Hendricks Research Foundation, NS25512, AG03951.

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10.3 SEROTONIN RECEPTOR GENE EXPRESSION IN THE RAT SUPRACHIASMATIC NUCLEUS. A.L. Rous*, D.R. Weaver and S.M. Reppert. Laboratory of Developmental Chronobiology, Children's Service, Mass. General Hospital, Boston, MA 02114.

The suprachiasmatic nucleus (SCN) receives a serotonergic (5HT) projection from the midbrain raphe nuclei. Quinpirole phase shifts the SCN circadian clock in vivo in the presence of tetrodotoxin, suggesting that a neuropeptide in the cells in the SCN express 5HT receptors (Brain Research 573:316, 1992).

We used in situ hybridization to examine the expression of 5HT receptor subtypes in the rat SCN by film autoradiography. Rat serotonin receptor cDNAs were kindly provided by O. Civel (5HT-1a) and D. Julius (5HT-1c, 5HT-2). Full-length 35S-labeled cRNA probes for the 5HT-1a, -1c, and -2 receptors, while cRNA probes for the 5HT-1b and -1d receptors were generated from cDNA fragments cloned using PCR. 5HT-1c receptor mRNA showed intense hybridization in the SCN, as well as in caudate-putamen and choroid plexus. The mRNA for 5HT-1b receptor also displayed a consistent, though weak, signal in the SCN, and a stronger signal in the hippocampus, caudate-putamen, and thalamus. 5HT-1a and 5HT-2 receptor mRNAs were not readily detected in the SCN, even though an intense signal was seen in other brain regions. The 5HT-1b receptor mRNA was not detected in the SCN or anywhere else in the brain. There was no obvious circadian variation in signal intensity for any of the examples we examined. We conclude that 5HT-1c and -1b receptor mRNAs are the most highly expressed 5HT receptor subtypes in the rat SCN.

10.5 NMIA RECEPTORS IN RODENT SUPRACHIASMATIC NUCLEUS. M.D. Hamburg, S.M. Grady and J.L. Fuchta. Dept. Biological Sciences, University of North Texas, Denton, TX 76203.

NMIA receptors appear to be involved in mediating effects of light on circadian rhythms. Pharmacological studies indicate that glutamate may be a transmitter in the pathway from retina to suprachiasmatic nucleus (SCN), the pacemaker of the circadian system. APV injected into the SCN diminishes light effects on the pineal (Ohi et al., 1991) and systemic MK-801 injections can block effects of light on activity rhythms (Colwell et al., 1990) and on food intake (Watanabe et al., 1990).

The present study aimed to characterize NMIA receptors in the rat and hamster SCN. In addition, 34 rats were used to test for changes in NMIA binding which may contribute to the phase-dependency of light effects: comparisons were made between 4 time points in LD, and between L versus D near dawn or dusk. NMIA binding was also measured in 10 encaged rats. Film autoradiographs were prepared from sections incubated with the NMIA antagonist [3H]MK-801 (Monahan '91). In both species, [3H]MK-801 binding was moderate in the SCN relative to other brain regions, was fairly uniform across the SCN region, and did not delineate the SCN. Scatchard analyses in SCN revealed similar binding properties in rats and hamsters. ANOVAs showed significant differences among treatment groups. The results demonstrate the presence of NMIA binding sites in the SCN; these binding sites could mediate NMIA antagonism-sensitive effects of light on rhythms. The absence of obvious changes attributable to enucleation, light condition or circadian time, suggests that in contrast to the phase-dependency of light effects on circadian rhythms, NMIA binding in the SCN is quite stable. Supported by NIH grant MH41865.

10.7 PARALLELISM BETWEEN NOCTURNAL CHANGES OF PLASMA MELATONIN AND TESTOSTERONE CONCENTRATIONS IN NORMAL, MALE ADULTS. TS. Schultz*, F. Chardon, M. Hugon and H.W. Rust. Division of Clinical Psychopharmacology, Department of Psychiatry, Geneva University, UHP, CH-1225 and Laboratory CNS, CH-1205, Geneva, Switzerland.

Differences or similarities in the temporal organization of hormones secretion in plasma reflect, among other phenomena, the activity of CNS pacemakers. We report on the simultaneous analysis of melatonin and testosterone concentrations in five normal male subjects between 23 and 32 years old were studied during 2 non-consecutive nights. Blood was withdrawn every 20 min during 12 hs, from 8:00 P.M. to 8:00 A.M. Testosterone concentrations during the night ranged from 1 to 10 ng/ml and melatonin from 200 to 2000 pg/ml. The mean concentration of testosterone increased 1.5- to 2-fold during the second part of the night. For melatonin, this increase was 2.5- to 4-fold. Rhythms of melatonin and testosterone were similar. This was to the point of high synchrony in 5 of the 10 nights: the times of onset of secretion, as well as the slopes of increase in concentrations were the same for the 2 hormones. This positive relation between melatonin and testosterone nocturnal rhythms is in apparent contrast with the inhibitory action of melatonin on the reproductive system of several mammal species. Dr. A. Andrei provided an antiseraum for the assay of melatonin. This study was financed by grant No 3.811.0.87 from the Swiss national research foundation.

10.8 ROLE OF PROLACTIN AND TESTOSTERONE IN SEASONAL PHYSIOLOGICAL CHANGES IN COLLARED LEMMINGS. R. Gower*, T.R. Nagy and M.L. Stacey. School of Life and Health Sciences, Univ. of Delaware, Newark, DE 19716.

Collared lemmings, Microtus montanus, display several interesting physiological changes on a seasonal basis. During autumn and winter, or when exposed to an artificial short photoperiod, lemmings store or in body mass, and develop a bifold "digging" claw. To determine the hormonal basis of these changes, we manipulated endogenous levels of prolactin (PRL) and testosterone (T) in animals exposed to short and long photoperiods. In animals transferred to 8L:16L, treatment with the dopamine agonist CB-154, treated with the dopamine agonist CB-154, and treated with the dopamine agonist CB-154. CB-154 treatment had a negative effect on testes weight in intact animals, but had no effect on the other parameters. Castration had a positive effect on body mass and growth in intact animals, but had no effect on the other parameters. Castration had a positive effect on body mass, and testes weight in intact animals, but had no effect on the other parameters. Castration had a positive effect on body mass, and testes weight in intact animals, but had no effect on the other parameters. Castration had a positive effect on body mass, and testes weight in intact animals, but had no effect on the other parameters.
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For the first time in 1987, we reported that sleep is sexually dimorphic in mice; prenatally stunted males were the same pattern of males, but not females. Prenatal stress reduces testicular enzyme activity with a reduction in plasma androgen (Ward, 1984). In the present experiment we used androgen insensitive testicular feminized (Tms) mice to investigate whether androgen receptors are involved in this phenomenon. EEG and EMG electrodes were implanted in four groups of adult male Tms mice. Normal male (Ts/Ts), heterozygous females (Tms/Tm) and phenotypic female Tms males (Tms/y). Two sleep recordings were taken continuously for 4 days.

Results: (1) Normal males spend less time in paradoxical sleep (PS) than normal females (3.95 vs 4.65 min/hr; p<.05) (2) PS in Tms males is higher than all other groups (p<.05).

3) Daytime slow wave sleep is indistinguishable between groups.

Conclusions: (1) The sexual dimorphism of sleep is replicated in another mouse strain.

(2) Deficiency of androgen in causing genetic males to develop female sleep patterns.


Environmental photoperiod regulates puberty in male ferrets. Testicular growth is accelerated in ferrets that experience a transition from short to long days, or are raised in 24-hr artificial light. Day-night transitions are transduced by the duration of the nighttime rise in melatonin secretion. This circadian pattern of melatonin release is regulated by a neural circuit that includes the retinohypothalamic tract (RHT), suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), and superior cervical ganglion. In hamsters, the integrity of each of three structures is necessary for reproductive responses to photoperiod. In mice, the importance of the SCN for photoperiodic sex differences has been established, but other components of the circuit have not functionally verified. In this experiment, exo-noradrenergic neurochemical lesions of the PVN were made in short-day-housed weanling (7 wk-old) male ferrets by bilateral stereotaxic injections of N-methyl-D-aspartic acid (0.6 µl of 0.3M NMDA in artificial cist). The onset of pubertal testicular growth normally begins at about 18 wk of age in ferrets raised in short days. However, within 3 wk of the lesion, at 10 wk of age, mean testis width was significantly larger in ferrets with PVN lesions compared to ferrets with sham suture. At sacrifice (16 wk of age), mean testicular weight was greater in ferrets with lesions than in controls (2.6 ± 0.8 vs 0.7 ± 0.2 g; p<.05), as was luteinizing hormone pulse frequency (5.0 ± 1.6 vs 1.5 ± 0.7 pulses/hr; p<.05). Thus, pubertal activation of the reproductive system was accelerated by PVN lesions. These lesions result in reproductive responses that are similar to those induced in prepubertal ferrets by a short-to-long day transition. This is the first indication that the PVN is part of the photoperiodic time measurement system in a non-rodent species. Supported by HD26483 and HD09950.

510.11 THE EFFECTS OF ROTATING LIGHT SCHEDULING ON ESTROUS CYCLEDICITY IN RATS. W.L. Woloshin and D.J. McEachom* Biomedical Engineering and Science Institute, Drexel University, Philadelphia, PA 19104.

The activity patterns of females rats vary systematically with the days of the estrous cycle. Control of these sexual cycles is thought to involve a SCN-mediated circadian gate interacting with an ovarian developmental clock. Given the strong ties between the SCN and photic environmental cycles, we examined the effects of different LD cycles on estrous rhythms. Running wheel activity was monitored for 3 female groups of female Sprague-Dawley rats. All were initially exposed to LD 18:6 for 4 weeks. All rats showed a circadian period of near 24 hours and an estrous cycle of 4 or 5 days which was 1 continued under the same LD cycle for the remainder of the experiment, 13 weeks. Group 2 was exposed to rotating shift cycle which mimicked a typical human shiftworker's pattern, i.e., day, swing, and night shift which was 2 weekends per week. Group 3 was exposed to a rapidly rotating schedule of 8 hour advances every 2 days.

Preliminary results indicate that Group 1 maintained a 24-hour circadian cycle and an estrous cycle equaling 4 or 5 days. Group 2 animals constantly lengthened their circadian cycle to 25.2 hours and their estrous cycle to 5 circadian cycles. Group 3 animals displayed highly variable results; circadian cycles ranged from 24.2 to 25 hours and estrous cycles from 3 to 5 circadian cycles. We conclude that despite the speed of the 3-shift cycle, Group 2 animals entrained to the LD cycles. The consistent lengthening of circadian and estrous cycles in Group 2 and the discovery of estrous cycles equaling 3 circadian cycles (+7 hrs) in Group 3 suggest that the relationship between circadian and reproductive cycles in female rats is more complex than has been suggested.


Sleep is sexually dimorphic in rats: Males have more paradoxical sleep (PS) than females. However, it is not clear whether this sex difference is influenced by ovarian hormones and the 4-5 day estrus cycle. The present experiment examines this possibility.

Adult Male and female Sprague-Dawley rats were implanted with EEG/EMG electrodes. Sleep recordings were taken for 20-30 days females and 4 days (randomly distributed in the same period) in males and ovariocectomized females.

Results: (1) No sleep was measured in 0.66 min/hr) than females (2.98 min/hr; p<.02) in both daytime and nighttime; (2) the sex difference in daytime PS is not lost in females after ovarcectomy; (3) the sex difference in nighttime PS is due mainly to a decrease of PS during the proestrus phase (4.88 min/hr vs 1.83 min/hr; p<.003); (4) ovarcectomy eliminates the proestrus phase difference (2.06 min/hr); (5) PS is indistinguishable between sexes.

Conclusion: (1) Nighttime PS depends upon the activational effect of ovarian hormones. (2) Ovarian hormones during daytime do not effect the sex difference in PS.

510.13 SUPRACHiasmatic NUCLEUS (SCN) LESIONS INCREASE SUSCEPTIBILITY TO ACTIVITY-BASED ANOREXIA. E.Z. Stanley, L.E. Donahue, T.L. York, and C.J. Pals. College of William and Mary, Williamsburg, VA 23185; Christopher Newport University, Newport News, Va 23601; Eastern Virginia Medical School, Norfolk, VA 23501; & V.A. Med. Ctr., Hampton, VA 23666.

Suprachiasmatic hypothalamic nucleus (SCN) lesions eliminate a variety of circadian rhythms. Circadian rhythms are known to be perturbed in anorexia nervosa. We hypothesize that the relationship between exercise and AN we have been exploring activity-based anorexia (ABA) in the rat (1.5 h/day food; 22.5 h/day wheel access). We have previously found that constant bright illumination (LL) increases susceptibility to ABA. This experiment determined if SCN lesions have a similar effect. Circadian rhythms were disrupted in two ways, viz., LL and bilateral electrolytic lesions of the SCN, according to a 2 x 2 factorial design (SHAM x LL vs 12/12 hr light-dark illumination; LD). Following a postoperative recovery period, rats were subjected to ABA. Susceptibility to ABA was defined as the number of days to lose 25% of body weight. Analysis of ad lib food intake before and after surgery demonstrated that the distribution of feeding was successfully altered by both the SCN lesion and LL treatments. When exposed to ABA, the SCN-lesioned groups required the least number of days to reach the weight-loss criterion. The SHAM LL group required an intermediate amount and the SHAM LD condition required the most days. There were no differences in terminal wheel revolutions or food intake across groups. These data indicate that a disruption of light-entrainable rhythms increases susceptibility to ABA. This raises the possibility that disrupted circadian rhythms are a novel risk factor for anorexia nervosa.


Rats anticipate a daily mealtime by entrainment of circadian oscillators outside of the suprachiasmatic nucleus (SCN). Neural mechanisms of this oscillator system are unknown. The present studies tested hypotheses concerning the possible role of sensory afferents and cortical, limbic and basal forebrain structures in the generation and entrainment of food- anticipatory rhythms. Food anticipation, measured by wheel- running and food-bio activity was robust in rats sustaining (a) trigeminal nerve deafferentation, (b) complete ablation of the hippocampus and amygdala, their efferents and forebrain targets, (c) complete ablation of the nucleus basalis, septum and adjacent medial forebrain and preoptic areas, or (d) complete removal of the neocortex. Intact rats receiving the dopamine antagonists haloperidol and losartan (30 min prior to oral or intragastric feeding time showed some attenuation of anticipation and overall activity, but so did saline injected intragastric fed controls. These results do not support previous suggestions that limbic or dopaminergic systems involved in memory and reinforcement processes have circadian functions necessary for food anticipation. Supported by NSERC, Canada.

We demonstrate that in Siberian hamsters show impressive decreases in body weight, almost exclusively as body fat, during the first few weeks of short day (SD) exposure. This decrease in lipid stores is not accompanied by a significant decrease in food intake at this time. As an indication of sympathetic nervous system activity, and consequently lipid depletion, white adipose tissue (WAT) catecholamine (CA) content was measured in SD (LD) and SD-housed hamsters. Male Siberian hamsters were killed 5 wk following transfer to SDs. Epidymal WAT (EAT) and heart (control tissue) were harvested, and nonpolar bioactive dopamine (DA) and epinephrine (EPI) content was measured by HPLC with EC detection. SD exposure increased NE content (mg/g protein) in EAT, but not in heart. DA content was not affected by the photoperiod for either tissue. For both photoperiods, the content of EPI was greater in EAT than in heart. SD exposure increased EPI content in EAT compared with that of the LD controls. Conversely, EPI content in heart was greater in LDs than in SDs. These results suggest that the rapid body weight (fat) decreases occurring during the first few weeks of SD exposure may be due to an enhanced sympathetic nervous system drive on WAT. In addition, possible WAT in situ conversion of NE to EPI, or of EPI-containing neural input is suggested by the high EPI content in EAT. Supported by NIMH R01 MH 06841 and NIH DK 35254 to TIB.

PHOTOPERIODIC CONTROL OF BODY FAT REGULATION FOLLOWING LAPTOPYCTOMY IN SIBERIAN HAMSTERS. M. M. Mast and I. I. Barnets. Dept. of Biology and Psychology, Georgia State Univ., Atlanta, GA 30303.

Siberian hamsters decrease body weight, primarily as body fat, when exposed to short, “winter-like” days (SD). We have shown previously that the apparent ability to “regulate” total body fat is photoperiod-dependent. In this experiment, male hamsters were housed in long days (light:dark 16:8) or transferred to SDs (light:dark 16:8) for 22 wk. SD-housed hamsters became phototrophic, showing a significant decrease in body weight and body fat (gain) over ~20 wk of SD exposure. Therefore, we thought that surgical lipopectomized (LIPX) hamsters undergoing naturally-occurring body weight (fat) increases would exhibit an enhanced ability to show seasonally-appropriate body fat levels. Paired epididymal white adipose tissue (EAT) fat pads were removed from EATs or sham surgery was performed in LD hamsters and in SD hamsters exhibiting a ~6% weight loss across the 22wk SD period. Twelve weeks post-surgery some EAT regrowth was seen in both LD and SD EATX animals (47 & 23% of sham control values, respectively). Surprisingly, only LD EATX animals regained body weight and restored epididymal fat pads weight increases above sham values. These LD results suggest a seasonally-appropriate ability to regulate total body fat that is not seen in SD regenerating hamsters. This inability of SD EATX hamsters to regulate total body fat may reflect a permissive action of the gonadal steroids on fat accumulation, since their tests only partially were recastrated (mean paired weights~250mg). Testes of this size likely would be accompanied by relatively lower serum testosterone (T) concentrations. The possible role of T in the ability to regulate seasonally-appropriate body fat levels is currently being examined. Supported by NIMH R01 DA 06841 and NIH DK 35254 to TIB.

BIOLOGICAL RHYTHMS AND SLEEP VI


In mammalian (premammalian nuclei) act as the dominant pacemaker controlling circadian rhythms (CR). In rodents, 11C-deoxyglucose (2-3) studies have demonstrated, both in vivo and in vitro that SCN energy metabolism increases with high levels of glucose-6-phosphate utilization during subjective day. In SCN lesioned animals, locomotor activity CR can be restored by transplantation of fetal whole tissue containing SCN. In the present study, we examined if the phase of the CR in host and donor SCNs were examined using 2-3 deoxyglucose as an index. Host and pregnant hamsters were housed in opposite light/dark cycles. On the day of birth, whole tissue containing SCN from neonates was implanted into the IIIrd ventricle of adult in females. Locomotor activity was recorded under constant darkness. 2-3 was injected during inactive (CT14) and active (CT4) periods. On the first day after grafting, the day clock retained its phase indicating that isolation of the SCN from the fetal brain and implantation into the adult host animal did not disrupt circadian rhythmicity in the donor clock. From the 14th day after grafting, host and donor SCN were in synchrony, whereas the phase of the host animal. The results indicate that the host SCN sends a signal which is effective in resetting the grafted SCN, and vice versa. Further 2-3 injections at various intervals after grafting will help to determine whether a diffusible signal is involved in synchronizing two clocks. Supported by grants from INRA (J.S.) and APOFIR F9002 (R.S.).

CIRCADIAN RHYTHMICITY RESTORED BY RAT-TO-HAMSTER ANTERIOR HYPOTHALAMIC (AH) HETEROGRAPHTS IS NOT ABOLISHED BY INHIBITION OF ANTIGENIC RESPONSE TO RAT. P.J. Sollars and J.C. Eckardt. Department of Psychiatry, Univ. of Penn., Philadelphia, PA 19104.

We report that rat-to-hamster heterotransplantation of fetal AH tissue contains the suprachiasmatic nucleus (SCN) are able to restore circadian rhythmicity to lpr-lpr RAG mutant hamster hosts. To determine the extent to which the rat implant is responsible for generating the restored rhythmicity, we examined the circadian rhythms of the host hamster to the immunological rejection of the AH heterografts. In animals with the most robust restored circadian patterns of locomotor activity, this procedure failed to abolish or even to disrupt the rhythmicity. This suggests that the skin grafts did not induce a rejection, the implanted neural tissue, or that the observed rhythmicity arose from an oscillatory capacity remaining in the brain of the SCN-lesioned host. Histological evaluation of the damage to the host SCN and of the viability of the neural implant will be conducted to distinguish between these possibilities. Supported by MH47501 to GEB.

SUPRACHIASMATIC NUCLEUS TRANSPLANTS normalize ENTRAINMENT IN AGED TAU- MUTANT HAMSTERS. M. R. Hard and M. R. Ralph. Dept. of Psychology, Univ. of Toronto, Toronto, Ontario, Canada, MSS 1A9.

Fetal suprachiasmatic nucleus (SCN) transplants are able to restore circadian activity rhythms that have been eliminated by lesions. Using a period mutation in the golden hamster, tau transplants and microsurgery procedures, we have shown that tau hamsters have demonstrated that period (24 vs. 20 or 22 hours) is an endogenous property of the tissue grafts. Expresses the tau rhythm at least partially the host of the host SCN, and both donor and host rhythms appear simultaneous if the host SCN is partially ablated. The partial SCN lesion causes reduces changes in activity rhythms in short-term period. Reduced amplitude and fragmentation. Similar changes accompany aging in animals which may reflect a progressive cellular and molecular degeneration within the SCN. We have therefore investigated expression of fetal SCN grafts in intact, both and have found previously that grafts are capable of increasing total activity and altering the period of the host. In these experiments, tau mutants that had received wild-type grafts were placed into a 14:10 light-dark (LD) cycle to assess entrainment patterns. Fetal tissue blocks from wild-type animals were harvested and transplanted into hosts of different genotype using a standard protocol described elsewhere (Ralph et al., 1990). Preliminary results suggest that transplanted animals are able to entrain to a LD cycle. Normally, tau mutants are either unable to entrain to a LD cycle or they entrain with an abnormally advanced phase angle (Ralph & Menaker, 1988). However, mutant animals with wild-type implants exhibited entrainment patterns that are characteristic of the wild-type. These results suggest that the SCN graft is able to integrate functionally with the circadian system of the aged host. Supported by the Alfred P. Sloan Foundation and the Natural Sciences and Engineering Council of Canada.

ALTERATIONS IN NEURAL EXCITABILITY IN VENTROBASAL COMPLEX OF THE RAT ACCOMPANYING CHANGES IN STATE OF AROUSAL. G.A. Marks and J.R. Holcomb. Dept. of Psychiatry, University of Texas Health Science Center, Southwestern Med. Sch., Dallas, TX 75235.

Neurons of the thalamus are influenced by state of arousal. Transfer of information through the thalamus is subject to state-specific changes related to both inhibition and excitation. The absence of these inhibitory interneurons in many dorsal thalamic nuclei, including ventrobasal complex (VB), might result in overall excitability changes that could contribute to the studies for the consequences of altered inhibitory mechanisms. Spontaneous activity of VB neurons in the rat and in neurons in thalamic nuclei that contain interneurons in similar that demonstrates the high in both experimental groups and REM sleep in SW sleep. However, bursty discharge occurs in rat neuronal cells rather than the single spike discharge that characterizes the REM sleep in other thalamic nuclei. Inasmuch as bursting activity is associated with high excitability levels, we attempted to uncover whether the pattern of excitability across states of arousal in thalamic nuclei varied. Excitability of individual VB neurons was measured by the proportion of times that the neurons fired above threshold levels (SCN in SW) applied to the medial thalamic during different states of arousal. In the majority of VB neurons, high levels of excitability in waking and REM sleep and relatively low levels in SW sleep. These are the same excitability changes animals returned to sham body weight and into. We conclude that the altered discharge pattern of VB neurons in rat does not make for a major functional change in state-dependent information transfer through this thalamic nucleus.
111.5 A CONTROLLED WAVEFORM TRANSFER FOR BIOLOGIC TIME- SERIES. S. L. Schiff, Dept. of Neurosurgery, Children's Nat. Medical Center, Washington, D.C. 20010.

Fourier transforms have had wide application to detect periodic components in biologic time-series, and are most accurately applied to time-series with "stationary" mean values. When faced with non-stationary, erratic biologic signals, wavelet transformations permit one to continuously explore along a signal for meaningful patterns at progressively smaller time scales. Recent use has been made of pseudo-random number random-cluster to biologic time-series. In 2 dimensional maps of galaxy and asteriod distribution using wavelet transformations. This same control technique is here applied to 1 dimensional time-series. The method is developed and verified with transient periodic functions, using sine functions contained within Gaussian envelopes contaminated with added noise. Noise can be effectively filtered out using pseudo-random number time series as controls, and non-random clustering of points and periodically detected. The ability to detect fractal and chaotic behaviors is explored. The method is then applied to a set of experimental time-series of spinal cord reflexes that has been previously characterized as a linearly correlated stochastic process. This method is widely applicable to experimental data from many biologic systems.

111.7 ORIENTATION OF THE CIRCATID LOCOMOTOR ACTIVITY RHYTHM IN CRAYFISH. J. L. Fuenzalida-f.1, M. Miranda-Avila and 1. A. Fuenzalida-M. Dep. de Biologia Experimental, Facultad de Ciencias, UNAM, Mexico, D. F., 04510, MEXICO.

The aim was to investigate the temporal organization of locomotor activity during orientation in crayfish. Procambarus clarkii juvenile instars, aged between 10 and 120 days after hatching were individually housed in especially designed activity recording cages under constant temperature conditions. The animals were divided in two experimental groups (n=20). In a first group each crayfish was left in free running condition under darkness (DD) during 30 days. In a second group each crayfish was in DD free running for at least 30 days and afterwards changed to a light dark cycle (LD 12:12) during the next 20 days. In general the activity rhythms of juvenile instars were not nearly as robust as those expressed by adults, and activity onsets were not well defined. Only the 50% of the age group showed the ability to synchronize to LD cycle (+42.1 h). The juvenile crayfish 120 days old displayed a free running rhythm and shorter circadian activity rhythm (+21.7 h) and fully synchronised to the LD cycle (+23.7 h). This result, although preliminary seems to indicate that the expression of the overt activity rhythm in crayfish is related to the postembryonic development.

This work was partially supported by PAPIEC UNAM 90120 and CONACyT 39 P04.


This study aims to elucidate circadian rhythm in neurons. Per repeat sequence, which is a homologous repeat sequence (ACNGCNNGC exists in the clock gene of Drosophila). A fragment of the genomic DNA sequence per repeat was cloned from mouse liver DNA library, and was identified to be cpl2. Eleven cDNA recombinants were cloned with cpl2 from rat brain cDNA library. One of them was designated pR815, which contained per repeat. The temporal and spatial expression of genes hybridized with pR815 was examined in the adult rat brain by in situ hybridization. Hybrization signals were observed in almost all neurons. Fluctuation of the signals under the light-dark cycle was apparently observed in the suprachiasmatic nucleus; the hybridization signals were intense in the middle of the day, but became weak in the middle of the night. However, the signal stayed relatively constant in other brain regions. The signal was detected in some glial and ependymal cells in the day but few in the night. The present findings suggest that genes hybridized with the per repeat sequence may be involved in the circadian rhythm in the rat nervous system.


Circadian rhythms generated by the suprachiasmatic nucleus (SCN) are entrained to environmental light. Light information normally reaches the SCN through two visual pathways: a direct pathway (BRT) from the retina and an indirect pathway (GHT) from the intergeniculate leaflet (IGL). The mutant anophthalmic mouse is characterized by the absence development of the GHT in these animals has not been explored. In the normal mammalian system GHT terminals in the SCN are known to contain NPY. We have used light microscopic methods to examine the GHT in anophthalmic mice. Serial sections were stained with cresyl violet for study of cytoarchitecture or with immunocytochemical methods for localization of NPY. Our data demonstrate that the IGL is well developed in anophthalmic mice. Perikarya and fibers in the IGL are innervated for NPY, and based on a robust plexus of NPY fibers and terminals in the SCN. GHT develops in the absence of visual input. (Supported by a grant from the Whitehall Foundation).
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992

THURSDAY AM

BIOLOGICAL RHYTHMS AND SLEEP VI

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11.11

BIOCHEMICAL MODELING OF THE BUHLA CUGULIANA OCULAR CIRCADIAN CLOCK. Michael H. Roberts, 3rd. Dept. of Biology, Clarkson University, Potsdam, NY, 13699

The eyes of several marine snails contain circadian pacemakers that, through these clocks and the expression of light, drive diurnal and free-running rhythms. Although these clocks and behaviors have been extensively studied, no detailed model of the cellular mechanism regulating the circadian rhythm has been explicitly formulated. Based on recent inhibitor studies, we have proposed that the cellular mechanisms generating the circadian rhythms of marine snails may be similar (Roberts et al., 1992). In support of this proposal, a mathematical simulation derived from a model of the cell cycle (Tyeon, 1991) is used to model the molluscan circadian rhythm. Manipulation of rate constants in the model corresponding to protein synthesis and tyrosine kinase activity produces results which match the effects of protein synthesis and tyrosine kinase inhibitors on the phase and the period of the circadian rhythm. In addition, period stability in spite of rate constant manipulation can be observed, suggesting a mechanism for the temperature compensation of circadian rhythms. These simulations provide support to the hypothesis that circadian rhythms in the molluscan eye are generated by biochemical processes similar to those generating the cell division cycle. Supported by MS26727.

11.13

THE TRANSCRIPTION INHIBITOR, 5,6-DICHLORO-1-6-RIBOFURANOXYLGENZIMIDAZOLE (DRB) BLOCKS THE PHASE-SHIFTING EFFECT OF CGMP ON THE CIRCADIAN RHYTHM OF NEURONAL ACTIVITY IN RABBIT LENS. A.H. C. Long, Neur. & Sensory Sci, Univ. of Virginia, Charlottesville, VA 22902

A phase advance of the circadian rhythm of neuronal activity in the SCN is induced by subjecting light-exposed rats to a 12L:12D cycle. The medium in the brain slice chamber was replaced with fresh medium containing 5,6-7-DRB at different concentrations. The neuronal activity rhythm in SCN was measured by monitoring firing rates the following day. A two-hour pulse of DRB (100 μM) given at CT 13-15 had no detectable effect, however, when given at CT 3-7, CT 5-7, CT 9-11, CT 17-19 and CT 21-23, it produced either arrhythmia or multiple activity peaks. DRB (10 μM) had no effect at CT 13-15 and CT 17-19 but disorders the circadian rhythm when given at CT 5-7 and CT 9-11. Treatment with 5,6-7-DRB (100 μM/5 μM) during the subjective day at CT 17-18 caused 2.7-hr phase advance. This phase-advance effect was totally blocked in the presence of 10 μM DRB (CT 17-19). When a DRB pulse was given either before the 5,6-7-DRB pulse at CT 15-17 or 1 hr late at CT 18-19, the effect of the cGMP analog was not blocked. These results demonstrate: 1) the SCN circadian rhythm is insensitive to DRB treatment during early to middle subjective night but is susceptible to treatment at other circadian times; 2) DRB blocks the resetting effect of cGMP. They suggest that transcriptional events are involved in maintaining the circadian oscillator and that immediate early gene transcription may mediate the phase-shifting effect of cGMP. (Supported by PHS NS 22155).

11.15

HB BLOCKS QUINCAPINE-INDUCED PHASE ADVANCES OF THE MAMMALIAN CIRCADIAN CLOCK IN VITRO. R.A. Proctor, M.C. Heffer, and D.J. Millar. Dept. of Biological Sciences, University of Aberdeen, Garthdee, Aberdeen AB9 2UB, Scotland. C.U. 949305

The mammalian circadian clock located in the suprachiasmatic nucleus (SCN) produces a 24 hr rhythm in spontaneous activity when isolated in vitro. This clock can be phase-shifted by a variety of agents, including AMP, as a function of agent and the neurotransmitter quinaprine. cAMP analogs induce phase advances in the subjective day, while quinaprine induces daytime phase advances and nighttime phase delays. The daytime effect induced by the 5-HT, agonists 8-OH-DPAT and blocked by the 5-HT1 agonist NAN-190. Since 5-HT receptors stimulate cAMP production in some systems, we investigated whether the effects of quinaprine could be blocked by a 5-HT antagonist. A blocker of the 5-HT1, the 5-HT2 antagonist quinaprine (1 μM), a blocker of the 5-HT3 receptor, and a blocker of the 5-HT4 receptor, quinaprine (1 μM). The activity of scb cells was recorded extracellularly on day 2 and their firing rates were averaged into 2 hr intervals to determine the pattern of SCN neural activity. This rhythm was then compared to that seen in untreated slices. We find that, while HB has no effect by itself at CT 6, it blocks the phase advance induced by quinaprine (2.5 μM) in advance with quip. (N=2, vs 0.05 μM in advance with quip+HB, N=3). In contrast, preliminary results suggest that HB does not block quinaprine-induced phase shifts at CT 15. Other preliminary results suggest that HB alone induces significant phase-delays when applied at CT 10. These results suggest that the chain of events through which quinaprine phase-advances the SCN clock includes the following: bind to 5-HT1 receptor → incr. cAMP → incr. protein kinase A (This work was supported by NSRA fellowship 8909 to RAP and the Upjohn Company.)

11.16


The suprachiasmatic nucleus (SCN) of spontaneously hypertensive rats (SHR) contain higher levels of vasoactive intestinal peptide (VIP) mRNA than do the SCN of normotensive Wistar-Kyoto (WKY) controls. Since the SCN is the site of a putative circadian pacemaker, and VIP is hypothesized to play an important role in the control of circadian rhythms, we have investigated whether circadian control is altered in SHR. Previously, we have reported that the onset of activity occurs nearly 1.5 hrs earlier relative to lights off in SHR than in WKY controls. In the present study examined whether the difference in the timing of activity onset might result from differences in the free-running period of the underlying circadian pacemaker. The free-running period of the circadian activity rhythm in constant darkness was significantly shorter in SHR rats than in WKY controls. The free-running period was lengthened by constant light in both groups but remained comparable. Further in SHR rats, the SHRs were confirmed to have higher blood pressure than WKY controls. These data suggest that the circadian period may be related to the levels of VIP in the SCN. (Supported by AG09301).

11.12

ANISOMYCIN BLOCKS PHASE SHIFTS OF THE BUHLA CIRCADIAN PACEMAKER TO LOW EXTRACELLULAR Ca²⁺ PULSES. S.B. Subbaraman & G.D. Block. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901

Circadian rhythms have revealed 2 types of phase response curves (PRCs) which are shifted 12 hrs in time from each other. In Buitha, pharyngeal and extracellular levels of light, depolarizing seawater, CNG (pharynx) and depolarizing current injection (Buitha) yield PRCs with phase shifts in the subjective night (depolarizing-type PRC). Pulses of CAMP, hyperpolarizing seawater, serotonin (pharynx), hyperpolarizing current injection (Buitha), low extracellular Ca²⁺ (Buitha) and FRM-Fmamide (Buitha) yield PRCs with phase shifts in the subjective day (hyperpolarizing-type PRC). It has been hypothesized that the potential current rhythm drives a transmembrane Ca²⁺ flux rhythm, and that these agents all phase shift by potentiating Ca²⁺ flux. Depolarizing-type PRC agents phase shift by generating a Ca²⁺ influx during the subjective night when it is normally low; hyperpolarizing-type PRC agents phase shift by inhibiting Ca²⁺ influx during the subjective day when it is normally high. Studies in Apis have shown that serotonin and CAMP induces phase shifts are blocked with anisomycin. This study addresses whether a similar blockade can be observed in Buitha for hyperpolarizing-type PRC agents. Pulses of low Ca²⁺ seawater (with EGTA) were applied in the late subjective day from CT 8-14 to yield phase advances relative to controls. When this experiment was repeated with additional 0.75 μM anisomycin applied to both experimental and control eyes from CT 7-14, no phase shift was apparent. Anisomycin applied alone at this phase has little effect. Preliminary data suggest that FRM-Fmamide-induced phase advances may also be blocked with anisomycin. These data provide support for the hypothesis that agents generating a hyperpolarizing-type PRC act via a common mechanism: inhibition of the transmembrane Ca²⁺ flux, which in turn generates a phase shift via a protein synthesis-dependent mechanism. (Supported by NS55264 to G.D.B.)
511.17
DIURNAL RHYTHM OF GALANIN-LIKE IMMUNOREACTIVITY IN HYPOTHALAMIC NUCLEI OF THE RAT. A. Alababiyi,1 J.J. Coppie,1 J.T. Alexander,1 S.E. Krykouli1, and S.F. Lebowitz.1 The Rockefeller Univ., NY, NY 10021 and Georgetown Univ. Sch. Med., Wash DC 20007

Galanin (GAL) is found to be abundant in the hypothalamus and has a range of functions in the physiological control of various endocrine and behavioral processes. The purpose of this study is to examine the diurnal rhythm in hypothalamic GAL levels to explore possible new roles for this brain peptide. Male Sprague-Dawley rats, maintained on a 12:12 hr light/dark cycle, were sacrificed by decapitation at light (N=10) and dark (N=10) onset. Ten hypothalamic nuclei, and the anterior (AP) and posterior (P) regions were examined. GAL was measured by RIA. Circulating serum corticosterone (CORT), aldosterone (ALDO), insulin, and glucose levels were also determined.

GAL levels were significantly higher at dark onset relative to light onset in the suprachiasmatic nucleus (24.5 ± 4.4 vs. 10.9 ± 1.8 ng/ml protein in the light, p<0.02) and the supraoptic nucleus (30.7 ± 4.3 vs. 15.3 ± 2.0 ng/ml protein, p<0.01). While a reverse trend, higher GAL levels at light onset, was apparent in the magnocellular paraven-tricular nucleus (PVN; 25.0 ± 5.1 vs. 19.1 ± 4.6 ng/ml protein, p<0.1), other areas (paraventricular PVN, arcuate nucleus, AP, and P) exhibited no diurnal rhythm of GAL levels. Measurement of circulating levels of hormones revealed strong diurnal light/dark rhythms for CORT and ALDO, with peak levels at dark onset. No significant relationship between these hormones and GAL levels was detected. These results indicate possible roles of GAL in biological circadian rhythm.

511.18
EFFECTS OF DESIPRAME EXPOSURE ON CIRCadian rhythms in RATS. A.M. Rosenwasser1 and M.J. Hayes.2 Dep’t of Psychology., Univ. of Maine, Orono, ME 04469.

Rats exposed to desipramine during early postnatal development show neuro-behavioral alterations in adulthood that may model human depression. In the present study, we applied this model to the study of free-running circadian drinking rhythms. Neonatal rats were cross-fostered and cycled into litters. Male pups were given daily desipramine (5.0 mg/kg, s.c.; N=6) or saline (N=6) injections on postnatal days 8 through 22. After the neonatal period, animals were exposed to a 12:12 hr light/dark cycle, with free access to water. Male Sprague-Dawley rats, maintained on a 12:12 hr light/dark cycle, were sacrificed by decapitation at light (N=10) and dark (N=10) onset. Ten hypothalamic nuclei, and the anterior (AP) and posterior (P) regions were examined. GAL was measured by RIA. Circulating serum corticosterone (CORT), aldosterone (ALDO), insulin, and glucose levels were also determined.

GAL levels were significantly higher at dark onset relative to light onset in the suprachiasmatic nucleus (24.5 ± 4.4 vs. 10.9 ± 1.8 ng/ml protein in the light, p<0.02) and the supraoptic nucleus (30.7 ± 4.3 vs. 15.3 ± 2.0 ng/ml protein, p<0.01). While a reverse trend, higher GAL levels at light onset, was apparent in the magnocellular paraven-tricular nucleus (PVN; 25.0 ± 5.1 vs. 19.1 ± 4.6 ng/ml protein, p<0.1), other areas (paraventricular PVN, arcuate nucleus, AP, and P) exhibited no diurnal rhythm of GAL levels. Measurement of circulating levels of hormones revealed strong diurnal light/dark rhythms for CORT and ALDO, with peak levels at dark onset. No significant relationship between these hormones and GAL levels was detected. These results indicate possible roles of GAL in biological circadian rhythm.

512.1
A PARADOXICAL SLEEP WINDOW FOR PLACE LEARNING IN THE MORRIS WATER MAZE. G.M. Rose* and C. Smith. Medical Research VAMC, Denver, CO, U.S.A. and Dept. of Psychology, Trent University, Peterborough, Ontario, Canada

Paradoxical (PS) sleep or REM sleep, deprivation is known to disrupt learning in both human beings and laboratory animals. Recently it has been shown that loss of PS during distinct periods, termed PS or REM windows, is as effective in inducing learning impairments as is total PS deprivation. In rats, PS is accompanied by rhythmic slow activity (“theta”) rhythm) in the hippocampus. Electrical stimulation patterned to mimic this theta rhythm is very effective in inducing long-term potentiation (LTP), a lasting increase in synaptic strength which is thought to serve as a memory encoding device. Taken together, these observations suggest the possibility that endogenous plasticity mechanisms are engaged during the PS window. However, a necessary step in verifying this hypothesis involves demonstrating the existence of a PS window for a hippocampus-dependent learning task.

Long-Evans rats were given 4 trials/day in the place learning version of Morris water maze. The rats were then selectively deprived of PS for a specific 4-hour interval after training. It was found that PS deprivation during the window 5-8 hours after training, but not at other intervals, delayed the learning of the location of the hidden platform. Thus, PS during a particular period after training is necessary for normal acquisition of this hippocampus-dependent place learning task. Studies are currently in progress to evaluate whether NMDA-receptor antagonists, administered during the PS window, will also retard place learning in the water maze.

512.2
CORONARY FLOW SURGES ENHANCED DURING THE PHASIC MUSCLE TWITCHES OF REM SLEEP. L.W. Dicker, D. Menard, M. Thannher, R.L. Yerger.1 Georgetown University School of Medicine, Department of Pharmacology, Washington, DC 20007

Previous studies in dogs showed dramatic increases in coronary blood flow (CBF) coupled with episodes of claus tachycardia during REM sleep. The present report found that 90% of these surges were concentrated during periods of phasic REM sleep and only 10% in tonic REM sleep. The incidence of these surges was related to the degree of phasic eye movement activity, that is, the surges were three times more frequent during intense phasic REM sleep than during moderately phasic REM sleep. However, the magnitudes of heart rate and CBF surges were unaffected by the particular subtype of REM sleep in which the surges occurred.

An additional enhancement of the magnitude of CBF surges was associated with the presence of phasic muscle twitches and not with frequency of eye movements or PGO waves. The increase in CBF (39.3% ± 1.8%) was significantly greater (p=0.006, unpaired t-tests) in phasic REM sleep with muscle twitches (n=100 events) than in phasic REM sleep without twitches (31.6% ± 1.8%) (n=62 events). There were no changes in blood pressure in either group and no significant differences in the magnitudes of the increases in heart rate between these two groups.

CONCLUSIONS: (1) A mechanism other than enhanced cardiac metabolic demand (HRxSBP) is implicated in the additional 8% increase in CBF concomitant with muscle twitches during REM sleep. (2) The tachycardia-associated surges in CBF represent part of the repertoire of autonomic responses intrinsic to the phasic periods of REM sleep in dogs. (3) The phasic events of REM sleep (eye movements v. muscle twitches) affect the incidence and magnitude of CBF surges differentially.

512.3
SLEEP APNEA PATTERNS IN LEAN AND OBSESE ZUCKER RATS. S. De Mesquita*, K.A. Burgess and F. A. Schone. Dept. of Physiology, Marshall University School of Medicine, Huntington, WV 25755-9340.

Sleep apnea is strongly associated with obesity in humans. This study investigated the frequency and type of sleep apneas in obese and lean Zucker rats. Nine lean (475±6g) and six obese (743±20g) Zucker rats were implanted with EEG and EMG electrodes and allowed to recover. All rats were monitored for sleep-wake patterns for 4 hr on five consecutive days. Prior to each sleep recording each rat was fitted with a chest pneumograph. 12 sets of apneas were identified: Quiet Apneas (QA) and Augmented Apneas (AA).

<table>
<thead>
<tr>
<th>Type</th>
<th>AAC (n=10)</th>
<th>ROC (n=10)</th>
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<tbody>
<tr>
<td>Obese (460±52)</td>
<td>2.2±1.4</td>
<td>1.7±1.1</td>
</tr>
<tr>
<td>Lean (280±42)</td>
<td>2.6±2.3</td>
<td>1.8±1.2</td>
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Two types of apneas tend to be longer in the lean rat during NR (Non-Rapid Eye Movement) sleep. The frequency of apnea occurrence was not significantly different between the lean and obese rats, however there were significantly more apneas during REM than Non-REM sleep.

512.4

Cerebrospinal fluid (CSF) from sleep deprived (SD) animals and VIP has been shown to increase REM sleep periods in normal and insomnic animals. The aim of this study was to determine the effects of CSF extraction (EXT) from SD cats on REM sleep rebound and to quantify by RIA the concentration of the VIP-1S in the CSF from SD cats. 43 adult cats implanted with a cannula in 4th V were used. EXP I: 23 cats were additionally implanted with electrodes for standard sleep-wake cycle recordings and were studied under the following conditions: control (CC), under SD by the water tank method for 24, 48 and 72 h and under SD for 24, 48 and 72 h with EXT of 100 ul of CSF. They were recorded during 12 hours. EXP II: 20 cats were studied in the following conditions: CC, under SD for 24, 48 and 72 h and CC of SD with a large platform (LP), for 24, 48 and 72 h. After the SD or CC, CSF (250-400 ul) was withdrawn. Then, all samples were analyzed with VIP 125 I RIA. Results showed that SD produces an increase of REM sleep which was abolished. CSF EXT of VIP this rebound is related to an increase in the concentration of VIP in the CSF from SD cats and this effect was independent of stress. These results suggest that during SD, the VIP is accumulated in the CSF and this may be causally related to REM sleep rebound.
12.5 EFFECTS OF CHRONIC SLEEP DEPRIVATION ON CENTRAL ADRENERGIC IN Contrast to the effects of sleep deprivation, which reduces REM sleep and increases waking, a similar effect was noted for hypothalamic sites with the highest activity of the hypothalamic site. The high activity of the hypothalamic site is correlated with sleep loss in the SD rats. Thus, the present results do not support a functional role of sleep in the upregulation of adrenergic receptors as proposed by Segel and Rogawski (Brain Res. 372, 213-233, 1988). However, the upregulation of hypothalamic α2 receptor binding sites after prolonged sleep loss suggests a possible role of hypothalamic adrenergic receptors in sleep-related mechanisms and functions.

12.6 HUMAN AND RABBIT INTERFERON INDUCE SLEEP IN RABBITS. M. Kimara-Takahashi, J. A. M. Deane, L. A. T. M. R. Oop and J. M. Krueger, Univ. of TN, Memphis, TN 38163, and Office of Naval Res., Arlington, VA 22217. Interferon (IFN) is usually considered a sleep-promoting substance since it has many other biological activities. Human recombinant (hu-r) IFNγ enhances rapid-eye-movement (REM) sleep in rabbits and rapidly reduces latency to REMs in monkeys. Patients undergoing IFN therapy complain of sleepiness. The somnogenic effects of IFNγ have not hitherto been documented nor have any species-specific IFNγ been asayed for their sleep promoting activity in the species of origin. Thus, hu-rIFNγ and rabbit (rb) IFNγ were tested in male rabbits implanted with EEG electrodes, a brain thermistor and intracranial cannula. Hu-rIFNγ received pyrogen-free saline solution on day 1 and, of 3 IFN preparations on day 2: hu-rIFNγ (Berlex Labs., Inc., 300000 or 1,125,000 U), rb-IFNβ (LEE Bioresearch Ltd., Inc., 400,000 or 800,000 U). Sleep-waking activity was monitored for 6 h after each injection. The high dose of hu-rIFNγ, but not the low dose, significantly increased NREM sleep and brain temperature (Tb), neither dose altered REMs. All doses of rb-IFNγ enhanced NREM sleep and elicited fever; none affected REMs. Further, in an antiviral assay using rabbit strain-13 cells, hu-rIFNγ was much less potent than the rabbit IFNγ. In conclusion, data suggest that the potencies of IFNγs are relatively species-specific.

Supported partially by ONR N00014-90-J-1069, NS 26429.

12.7 SLEEP DEPRIVATION INCREASES CATHELAMINE TRANSPORTER AND TYROSINE HYDROXYLASE TRANSECTION IN THE RAT. E. A. Bergman, T. T. Fiebig, J. E. H. Funder. Dept. of Physiology, Univ. of Basel, 4056 Basel, Switzerland. Bascom, CA 93306-3320. Adult male rats were deprived of REM sleep (RMS) for 6, 24, or 72 hours, or were allowed to sleep for 6 or 24 hours after 24 hours of deprivation. The deprivation group (DF) was kept on small platforms (diameter 6.5 cm) in a water bath, while the control animals were kept on large platforms (diameter 12 cm) or in a dry cage (DC). In the rats the hypothalamus was rapidly dissected and frozen for catecholamine measurement while in other rats the whole-brain was removed for measurement of tyrosine hydroxylase (TH) RNA. Catecholamines were measured by high performance liquid chromatography with electrochemical detection. The TH/NA ratio was significantly higher in the posterior hypothalamic (PH) of DF animals after 24 hours of deprivation (p<0.05) and significantly lower after 24 hours of recovery sleep (p>0.05). Norepinephrine was significantly increased in the PH after 72 hours of deprivation (p<0.05). TH RNA was significantly increased in the locus coeruleus in the PH after 72 hours of deprivation (p<0.05). These findings suggest that catecholamine function is enhanced during REM deprivation.

12.8 RELATIONS BETWEEN REM SLEEP AND THE PONTINE AUDITORY PI EVOKED POTENTIAL INDUCED BY CHOLINERGIC AGONISTS INJECTED INTO THE PONS. Z. Eliazyt and Y. Naval. Dept. of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv 39578, Israel. PI potential, the 20-30 msec mid latency component of the vertex auditory evoked potential, was found to decrease in slow wave sleep and increase in wakefulness and REM sleep (Chen & Buchwald, 1986). We recorded this potential from the posterior part of the cuneus chronically prepared with electrodes and cannulas for sleep studies. The pontine PI potential was similar in shape but of shorter latency than that recorded from the vertex. The amplitude varied with the behavioral state and was always higher in REM sleep than in slow wave sleep. The latency increased with age and the latency varied. The PI potential was investigated to understand the relationship between the PI potential and the SWS and REM sleep. The amplitude of PI decreased gradually to reach the level of the natural potential after 60 to 120 minutes, consistent with the degree and time course of REM sleep enhancement. These results suggest that the amount of REM sleep is related to the intensity of cholinergic synchronous activation of pontine neuronal populations expressed by potential PI.

12.9 IL-18 ANTIBODIES REDUCE SLEEP AND ALTER IL-18 INDUCED ENHANCEMENT OF SLEEP IN THE RAT. Mark A. Ong & James M. Krueger. Dept. Physiol. & Biophys., Univ. of TM, Memphis, TN 38163.

Interleukin-1 (IL-1) is a cytokine with pleiotropic biologic actions (1). It is hypothesized that IL-1 is involved in the physiological regulation of sleep (2); if this is the case, then reduction of IL-1 levels at appropriate receptors should result in loss of sleep. To test this hypothesis, male Sprague Dawley rats were injected with a monoclonal (ICV) injected with 3 doses of anti-IL-18 antibody (Cytokine Scilance; 5, 10, 20 μg) at light onset and sleep-wake acivity was determined. The amount of time spent in non-rapid-wy movement sleep (NREM) or in REMs was not greatly affected by the dose of anti-IL-18. However, after the 20 μg dose of the antibody, the amount of time spent in NREM was reduced by 50% across the 3 doses. However, the REM was not reduced; REMS was not greatly affected. To determine if anti-IL-18 would antagonize the effects of exogenously administered hu-rIL-18, another group of rats was probed with ICV anti-IL-18. After 1 dose of "lights off" and injected just prior to "lights on" with 10 ng hu-rIL-18, the characteristic enhancement by hu-rIL-18 was abolished or reduced. These data support the hypothesis of a role for IL-1 in sleep regulation. Supported in part by NS 25378 and MH 47103. 1) Dinarello, CA. Blood 77, 1657-1667, 1991. 2) Krueger, J. et al., Yale J. Biol Med 63:1571-1572, 1991.


Adrenergic input to the medial pontine reticular formation (mPRF) has been postulated to play a role in the control of REM sleep in the slow-wave sleep. We have previously reported (Soc. Neurosci., Abst., 1991) the effects on sleep-wake states of adrenergic antagonists norepinephrine (NE, mixed α1,α2 agonist), phentolamine (PHE, an α1 antagonist) and clonidine (CLON) microinjected into the mPRF. These data showed that CL is REM suppressive, presumably by action at α1 receptors. The REM suppressive effect was localized to those regions of the mPRF which give short latency, long duration increases in REM following infusion of carbachol. Here we describe the effect of α1 antagonist idazoxan (IZ) on REM sleep. A series of microinjections into the mPRF of 12 (55 mmol and 550 mmol in 0.5 μl / 1 minute) were performed. In the first hour REM sleep was decreased following IZ (55 mmol, - 38.9% : 550 mmol, - 51.9%). In the second hour IZ at a dose of 55 mmol reduced REM by 56.7%, but the higher dose of 550 mmol increased REM by 44.1%. In the third hour REM sleep was increased at both doses of IZ (55 mmol, 24.4% : 550 mmol, 40.0%). In the fourth hour IZ increased REM at both doses (55 mmol, 25.0% : 550 mmol, 62.5%). Across the entire 4 hours of recording REM sleep was significantly increased by IZ at 55 mol (44.7%; n=12; p=0.05) while the higher dose also produced an increase (155%), but this effect was not significant with 550 mmol. These data indicate a REM-enhancing role of IZ. This action is presumably mediated through antagonism of α1 receptor activation but heterogeneity of this receptor and binding of IZ to imidazoline sites pharmacologically distinct from the putative interpretation of action in-vivo. SUPPORTED BY NIHMS Grant MH18825.
S12.11

EFFECTS OF A LOW DOSE OF 8-OH-DPAT ON SLEEP IN THE RAT. BS Pastele* and P Covington. Department of Medical Neuroscience, Walter Reed Army Institute of Research, Washington, DC 20307.

8-OH-DPAT (8-hydroxy-2-(1n-propylamino) tetralin hydrobromide) is a selective and potent 5-HT1A agonist. One previous study demonstrated that 2.5 mg/kg of 8-OH-DPAT suppressed both rapid-eye movement (REM) and non-REM (NREM) sleep. However, that dose also induces the serotonin behavioral syndrome, hypothermia and increased feeding. We investigated the effects of 10 ug/kg of 8-OH-DPAT, a dose which does not produce these effects.

Three male rats were given saline (sc) and 24 hr later 8-OH-DPAT (10 ug/kg, sc). Treatments were administered at light onset and EEG was measured for 6 hr following injection. Administration of 8-OH-DPAT increased wakefulness and decreased both NREM and REM sleep. The suppression of REM and NREM sleep, reported previously using a 2.5 mg/kg dose, could have been due to the induction of the serotonin behavioral syndrome, feeding behavior and/or hypothermia. The present results, using a smaller dose of 8-OH-DPAT, suggest that the effects of 8-OH-DPAT on sleep may be due to an effect on 5-HT1A receptors.

S12.12

DISRUPTION OF LIGHT-INDUCED C-FOS IMMUNOREACTIVITY IN THE SUPRACHIASMATIC NUCLEI (SCN) OF MIDDLE-AGED FEMALE RATS. J.M. Lloyd, A. Cai and A. P. Wise. Department of Psychology, University of Maryland School of Medicine, Baltimore, MD 21201.

Mammalian circadian rhythmicity of various biological functions is maintained by a light-entrained pacemaker in the hypothalamic suprachiasmatic nuclei (SCN). Recent work suggests that light-entrainment may involve the induction of specific immediate early genes such as c-Fos. To determine if age-related deficits in SCN function involve altered levels of expression we compared C-Fos expression in the SCN of young and middle-aged animals. Regularly cycling or ovariecotomized estradiol-treated young (3-4 month) and middle-aged (10-12 month) animals were administered an overdose of pentobarbital on proestrus or after 2 days of estradiol and perfused 75-90 min before and after lights on. Brains were processed for immunocytochemical localization of c-Fos (Cambridge Research, OA-11-823). In agreement with previous studies, in young animals C-Fos expression was evident only after lights on. However, in middle-aged animals, (a) in some instances, expression of c-Fos can be observed prior to lights on, (b) the intensity of light-induced c-Fos expression appears to decrease, and (c) the number of C-Fos containing neurons decreases. These data demonstrate that there is a disruption in light-induced C-Fos expression in the SCN of middle-aged animals. Supported by NIH AG02254 and NIA AG05525.

S12.13


Light pulses which phase shift circadian rhythms also induce several immediate early genes, including c-Fos, in the suprachiasmatic nuclei (SCN, Kornhauser et al., Science, 253, 1992). Both carbachol (an acetylcholine agonist) and forskolin (an adenylyl cyclase activator) have been reported to phase-shift circadian rhythms. We used an in vitro slice preparation to determine if these pharmacological agents would also be associated with increased Fos-like immunoreactivity (Fos-lir) in the SCN.

Brain slices (600 μm) obtained from male golden hamsters housed under 1L:1D at either Zeitgeber Time (ZT) 0 or ZT 9 (ZT 12:00) were used. The slices were maintained as described previously (Harrington et al., Soc. Res. Biol. Rhythms abs. 1992). Carbachol (10 μM) and forskolin (24 μM) were applied 6 hr post-dissection. Immunohistochemistry was performed 10-12 min after each treatment. Thirty minutes later all slices were placed in cold 4% paraformaldehyde for 4 hr. After storage in 30% sucrose, brain slices were sectioned (30-45 μm) into cryoprotectant (2xP) and stored at -80°C with a rabbit polyclonal antibo (1:200) raised against the N terminal (2-17) of rat Fos (D. Hancock and G. Evan, ICRF, London).

Control slices showed little Fos-lir in the SCN. Carbachol was able to induce Fos-lir at ZT 14. Forskolin was able to induce Fos-lir at both ZT 6 and ZT 14. Photic induction of Fos in vivo is only observed in the subjective night. Our results indicate that this may be due to a circadian fluctuation antecedent to or not involving adenylyl cyclase activation. Supported by NIH NS32696 (MEB) and NIMH 44132 (EBL).

S12.14


Since it has been shown that intraventricular administration (IVA) of VIP induces a selective increase in REM sleep, we used Foslike immunostaining (FLI) for constructing maps of the pattern of second messenger activity in the brain stem, after REM sleep induction by this manipulation. Nineteen Wistar rats were implanted for conventional sleep recordings. Additionally, a stainless steel cannula was implanted into the 4th ventricle. Two groups were used: a control. In which an IVA of 5U saline was applied, and a VIP group where 100 mg of VIP in 5 μl was injected IVL. After 1hr of sleep, the animals were anesthetized and perfused with 4% paraformaldehyde. The brains were processed for immunohistochemistry with the ABC technique of Hsu et al., 1982. We used a Fos antibody provided by Tom Curran. The results showed an increase in REM sleep frequency in VIP group. Moreover, this group showed a significant increase in FLI in several brain stem structures. These results suggest that VIP sleep inducing properties may depend on an increment in the number of active neurons in a widespread network in brain stem.

S12.15


We sought to determine the time course of activation of the immediate-early gene, c-fos, in conjunction with rapid-eye movement (REM) sleep. Carbachol, a mixed cholinergic agonist, was used to produce a sustained REM sleep episode. Fos-like immunoreactivity was assessed at various times after the end of REM sleep.

Carbachol elicited 15 min to 70 min long REM sleep bouts. In carbachol animals examined immediately upon awakening at the end of carbachol-REM sleep, counts of Fos-lir cells in the raphe, LC, LT-PPT and the medial PRF were higher compared to vehicle controls. One carbachol animal with a 45 min REM sleep bout showed few Fos-lir cells 24 hr after end of REM sleep. A lower dose (0.2 μg/0.25 μl) of carbachol produced no REM sleep and no Fos-lir cells compared to vehicle controls. One carbachol animal had no REM sleep and no Fos-lir cells.

Fos-lir occurs in association with cholinergic-induced REM sleep in pontine regions implicated in REM sleep. A minimum duration of REM sleep appears to be necessary for Fos-induction. However, a plateau in number of Fos-lir cells was observed at 15-45 minutes of continuous REM sleep. Some of the Fos-lir cells are cholinergic or noradrenergic. Supported by DA Research Service, NS30140, and NS32722

S12.16


Many of the sleep disturbances of the elderly such as the fragmentation of the major sleep period and early morning insomnia may be due to a deterioration in the circadian control of sleep and wakefulness. In an effort to address this issue at the molecular level, we have focused on circadian aspects of immediate-early gene (IEG) expression, which may be involved in both input and output mechanisms of the circadian pacemaker. Two approaches have been utilized: 1) The photic induction of IEGs in the suprachiasmatic nucleus (SCN) was examined in young vs. old rats. In a pilot study, young (4 months) and aged (22 months) male Fischer-344 rats were given either a 30 min light pulse late in the subjective night at circadian time (CT) 22 or left in darkness. Message levels were identified by in situ hybridization using a 32P cRNA probe to c-fos. Aged rats that received photic stimulation at CT 22 demonstrated a blunted response of c-fos mRNA expression in the ventrolateral SCN relative to young rats. Neither young nor old rats remaining in darkness showed any circadian expression of IEGs. Expression in extra-SCN regions was investigated across the circadian cycle using Northern blot analysis. Nocturnal rats have the highest levels of c-fos message during the night while maximal levels were observed in dorsal ground squirrels during the day, suggesting a correlation with activity. In contrast, c-jun mRNA was invariant in the rat but increased during the active phase in squirrels. Surprisingly older rats, which have uniformly rhythms, a somewhat higher level of c-fos message was observed. In addition, two larger messages cross-hybridized with c-fos that were not apparent in the younger rats. Taken together, these results suggest alterations in circadian expression of IEG regulation which may help elucidate mechanisms underlying the age-dependent decay of circadian organization.

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S13.17
Sleep Research Center, Stanford University, Stanford, CA 94305.

The two-process model of sleep regulation postulates that a homeostatic drive to sleep, referred to as Process S, increases with time spent awake. The purpose of this study was to evaluate whether immediate early gene expression is increased in synchrony with time spent awake, when Process S would be expected to increase. Rats were deprived of sleep by gentle handling beginning at light onset for 45 min, 3 hr or 6 hr. The duration of sleep deprivation in each of the three groups was chosen to correspond to the previously described response of EEG delta power to sleep deprivation. At the end of the deprivation period, animals were sacrificed by decapitation, the brain dissected, and subregional and fronto-motor cortex c-fos expression was measured in all brain regions from all animals, however, the sleep deprived animals showed higher expression than the control animals at all time points. Curiously, the highest levels of expression were observed in the 45 min as well as 6 hr deprived rats. The high c-fos mRNA expression in the 45 min animals may be attributable to the transient stress of movement to the deprivation room. The high c-fos mRNA in the 6 hr animals may reflect either (1) increased c-fos expression in parallel with build-up of Process S or (2) increased expression due to stress induced by increased handling of the deprived animals. Preliminary results indicate that c-jun does not undergo the same changes exhibited by c-fos whereas jun-B is more similar to c-fos. Further efforts will be directed towards (1) other modes of sleep deprivation in an effort to minimize stress; and (2) examination of other IEGs (Supported in part by the Upjohn Company).

S13.18
Sleep Research Center, Stanford University, Stanford, CA 94305.

Changes in arousal state such as sleep and hibernation are likely to be accompanied by changes in gene expression, some of which may facilitate state transitions. The goal of this project is to evaluate whether gene expression is altered in specific brain regions across the hibernation cycle. Our initial focus is on immediate early gene (IEG) expression because IEGs are known to play a critical role in the regulation of long-term changes in gene expression. Golden-mantled ground squirrels (Citellus lateralis) were implanted with abdominal telemeters to monitor body temperature (Tb) continuously. Animals were placed in a constant temperature environment at 5°C under LD 12:12 photoperiod. Northern blots were then prepared for secondary hybridization with [32P] c-fos and c-jun probes. Expression of c-fos and c-jun show clear changes throughout the hibernation cycle. For example, both genes exhibit a rapid increase in message in the hypothalamus during arousal from hibernation. This observation is of particular interest because previous work, including our 2-deoxyglucose studies, has shown this brain region to play a critical role in the arousal process. Preliminary results from in situ hybridization studies suggest that the supraspinal nucleus is among the hypothalamic regions exhibiting this increase (Supported in part by the Upjohn Company).

S13.19

Light exposure during the subjective night activates the expression of several immediate-early genes (IEGs) in the suprachiasmatic nucleus (SCN) of the Syrian hamster and causes phase shifts in circadian activity rhythms. The role of these light-responsive cells in the circadian pacemaker in the generation of behavioral and endocrine rhythms is not known. We have combined retrograde labelling of SCN neurons with photic induction and immunocytochemical detection of Fos and Egr-1 in an attempt to determine whether induction of IEGs occurs in cells which project to identified targets of this nucleus or to the contralateral SCN.

Hamsters (n=10) maintained on a 16L:8D cycle received unilateral stereotaxic injections of 0.1-0.3uL of fluorescent latex microspheres (Lumanal, Inc) aimed at the septum, preoptic area, hypothalamo paraventricular, paraventricular nucleus area, intergeniculate leaflet (IGL), or SCN. After a survival period of 1-5.3 weeks, animals were exposed to light for 1h commencing 3 h after lights off and were then anaesthetized and perfused with 4% paraformaldehyde. Frozen sections (40um) were processed for Fos and Egr-1 immunoreactivity (i-r) and examined for fluorescent label.

Light exposure reliably induced Fos-i-r and Egr-1-i-r in the SCN and the IGL. As reported previously, the greatest concentration of IEG-i-neurons was seen in the ventrolateral SCN. Immunoreactive cells also occurred in the surrounding anterior hypothalamus. Retrograde labelling of SCN and subparaventricular neurons revealed projections to each of the areas targeted. Less than 1% of the Fos-i-r or Egr-1-i-r cells contained fluorescent microspheres. It is concluded that photic induction of these IEGs occurs principally in intrinsic cells of the SCN rather than its projection neurons. Supported by SERC (GR/H08716) and The Royal Society.

INGESTIVE BEHAVIOR: WATER AND SALT INTAKE

S13.1

Intragastric infusions of hypertonic solutions activate spinal thirst centers to increase plasma vasopressin without changing plasma osmolality in rats (Choi-Kwon & Berendes, Am. J. Physiol. 261: E16, 1991). We examined whether such infusions elicit drinking without systemic dehydration. Adult Sprague-Dawley male rats (n=32) were surgically prepared with a chronic gastric catheter. A test in rats not deprived of food or water was initiated by a 2 ml (in 1 min) infusion of 300, 600, 1200 or 1800 mM NaCl or sucrose, mannitol, LiCl or Na isethionate. The latency to initiate drinking was decreased (p<0.05) and 60-min water intake was increased (p<0.05) by 600, 1200 and 1800 mM NaCl compared to 300 mM NaCl. Plasma osmolality at initiation of drinking was not changed from baseline (300 mM NaCl) by 600 or 1200 mM NaCl but was increased by 1800 mM NaCl (p<0.05). Equiosmotic solutions of sucrose, mannitol, Na isethionate, LiCl and LiCl also elicited drinking. Drinking elicited by 600, 1200 or 1800 mM NaCl was abolished by total abdominal vagotomy (p<0.05). Our results are consistent with the hypothesis of a vagal-mediated osmosensitave gastrointestinal and/or hepatic-portal mechanism for eliciting drinking in advance of systemic dehydration in the rat.

S13.2

Water intake induced by either central or peripheral injection of Ang II is blocked by the AT-1 receptor antagonist losartan (Sandoz 753). We report the effects of acute cerebroventricular (ICV) injection of losartan (50 ug: 500ug the dose that blocks water intake to ICV Ang II) on various types of thirst in rats. ICV losartan had no effect on water intake following either hypertonic NaCl, isoproterenol, carbachol (ICV), or on food intake in a dessert test. ICV losartan blocked drinking caused by either SC injection or IV infusion of Ang II. The antidiuretic effect of central AT-1 blockade thus is selective for Ang II.

In contrast, ICV administration of the AT-2 receptor antagonist, PD 123319 (100ug) blocked water intake following injection of hypertonic NaCl, isoproterenol, ICV carbachol and Ang II. ICV PD 123319 did not reduce food intake in a dessert test. Thus, the AT-2 antagonist may have a common action on the various mechanisms of water intake, but it does not nonspecifically reduce food ingestion. We speculate that an AT-2 receptor may be on a 'final common pathway' for the integrated thirst signal. Supported by the American Heart Assoc., Florida affiliate.
513.3 ANGIOTENSIN RECEPTORS IN SFO BUT NOT OVL MEASURABLE ISOPRETERENOL-INDUCED DRINKING. D.A. Fitz*, Dept. of Psychology, Univ. of Wash., Seattle, WA 98195

Rats had chronically implanted cannulae in the third ventricle of the subfornical organ (SFO) drank an average of 0.2 ml water after isopretorenol, 10-50 mg/kg SC, compared with the 0.1 ml of water in ovine controls. The doses of isopretorenol were much lower than previously reported in SFO lesioned rats (160 μg/kg, Simpson et al., J.O.P.S., 1978), so this lesion-induced reduction to high doses of isopretorenol.

Another experiment compared the effects of 90 min infusions of the angiotensin (ANG) receptor blocker [Sar1, Thr3]-ANG II (0.45 μg/min) or angiotensin II into the ovine vasomotor nucleus terminals (OVT), or lateral ventricles after 20 mg SC isopretorenol. A sarathrin infusion into SFO significantly reduced drinking from 0.4 ml of water in ovine control conditions to 2.3 ± 0.6 ml (mean ± SEM, p < 0.01), the same infusion into OVT (465 ± 0.7 ml) or into the lateral thalamic ventricle (0.4 ± 0.5 ml) did not. Sarathir was ineffective at 0.1, 0.45 or 4.2 μg 4 ml each in SFO and 500 (3.3 ± 0.6 vs 4.4 ± 0.5 ml) pmol/ml into the lateral ventricles.

ANG accounts for at least 47% of the drinking observed after isopretorenol treatments in rats, and the receptors critical for this drinking are in SFO rather than OVT. The study also confirms that blood-side ANG receptors in SFO are responsible to moderate doses of ventricularely applied blockers (Fits & Masson, Behav. Neurosci., 1990). This explains some negative results in previous studies at the peripheral ANG from the ventricles.

Supported by NS-22274.

513.5 CHANGES OF DRINKING BEHAVIOR IN THE RAT AFTER SEPTAL LESIONS. R.-M. Liao* C.-C. Yeh; Dept. of Psychology, National Cheng-Chi University, Taipei, R.O.C.

The septum has been suggested to play an inhibitory role in the drinking behavior. It is also known that the septum is heterogeneous in terms of neuroanatomical perspective. The present study examined the water intake and the locomotor activity of rats lesioned with kainic acid (0.5 μg/0.5 ul/site) on three septal subregions: anterio-lateral (Msa), posterior, medial (Msp), and lateral (Ls) sites. There were significant differences between groups on both measurements in the postlesion tests. Drinking and locomotor activity was enhanced by the Msp lesion, so was the locomotor activity. Another experiment further determined the dopaminergic effects of intracranial glycine (PEG, 204, SC) and hypertonic saline (NaCl: 1M, IP) in the Msp lesioned rats. These cellular and extracellular thirst stimuli were given on the 10th postlesion day. Water intake was significantly increased by the hypertonic treatment, but not by the injection of PEG. In addition to showing the septal hyperdipsia, these data suggest that the septum is functionally heterogeneous in drinking behavior.

(supported by NSC 80-0412-B004-01)


In the intact sodium depleted rat, sodium intake is driven by the synergistic actions of angiotensin II (Ang II) and aldosterone on the brain. Removal of the adrenal glands results in a marked loss of sodium and an increased sodium consumption. Our previous study has shown that central administration of sarcosine (Sar) and angiotensin II (SARILIE), a non-selective Ang II receptor antagonist, suppressed sodium intake in aldosterone deficient male rats. The sodium intake was dose dependent and the highest dose of SARIEI resulted in a minimal intake, which was still present after aldosterone replacement suggesting that the residual intake was need-free intake. In the present experiment we show that blockade of AT1 receptors inhibits the sodium intake of adrenalectomized rats. Adrenalectomized female (n=6) and male rats (n=6) were given a 3% NaCl solution 2 hours per day, and received daily injections of desmethylamph (200μg) to compensate for the lack of corticosterone and a prophylactic treatment of gentamicin (40μg/day). On the day of the experiment, 15 minutes before the test rats were given 5% NaCl, the females were given a pulse intracerebroventricular injection of DaP 753, a selective AT1 receptor antagonist at doses of 300 μg/ml or 1000 μg/ml (females), 50 μg/ml or 1000 μg/ml (males), counterbalanced by an injection of 2 μl of saline vehicle. The intake of salt was recorded thereafter at 15 min, 30 min, 1 hour and 2 hours. At all doses of DaP, sodium intake was suppressed in both males and females. In those groups of both sexes the intake of salt was completely abolished in males, while a residual intake persisted in females at all doses of sodium intake. It is concluded that both female and male adrenalectomized rats, brain Ang activity is responsible for the increased sodium intake, and that AT1 receptors are involved in the neuroregulalation of the brain Ang activity.

Supported by NIH program project # MH-43878.


We have previously reported that intravenous (IV) infusion of aldosterone (ALDO) induces sodium intake in rats. Here we studied the role of central mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) in mediating this ALDO-induced sodium intake. Male rats were fitted with an intracerebroventricular (ICV) cannula and an IV catheter. Water, 3% NaCl and Purina Chow were available ad libitum. Rats received a continuous ICV infusion of the Type I, MR antagonist, Ru-28318 (10μg/hr) combined with concurrent IV infusion of ALDO (3 μg/hr). Central administration of Ru-28318 completely blocked ALDO-induced 3% NaCl intake (iV ALDO = 9.5±3 μl vs iV ALDO + ICV Ru-28318 = 0.4±0.2 μl 3% NaCl). The suppression of ICV ALDO-induced 3% NaCl intake was reversed once the ICV Ru-28318 infusion was stopped. Continuous ICV infusion of the Type II, GR antagonist, Ru-38486 (10μg/hr) was ineffective in suppressing ALDO-induced 3% NaCl intake. Together, these data provide evidence for a role of brain Type I MR involvement in ALDO-induced sodium intake in the rat. Supported by MH 43787 and NSO 3469.

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INGESTIVE BEHAVIOR: WATER AND SALT INTAKE

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S13.9

BRAIN SODIUM AND SODIUM APPETITE IN THE RAT. S.Y. Chow, A.P. Epstein, B.S. McEwen, S.J. Flaherty and R.R. Sakai. Univ. of Pennsylvania, Philadelphia, PA 19104 and The Rockefeller University, New York, NY 10021. We examined the effects of modifying brain cerebrospinal fluid (CSF) sodium concentration during sodium depletion on the expression of sodium appetite in the rat. Male rats fitted with intracranial cannulae were sodium depleted by combining treatment with furosemide and removal of all ambient sodium. They received either 2M NaCl, 0.7M mannitol or isotonic saline (5mM Na) continuously infused 1h before the beginning and throughout the depletion treatment. Sodium was measured by urine collection for 24h preceding and the following day. Infusion of 2M NaCl decreased 3% NaCl intake (5.7 ± 1.9 ml/s) and intake of 0.7M mannitol increased 3% NaCl intake (12.4 ± 1.2 ml/s) as compared to injection of isotonic saline (9.4 ± 1.9 ml/s). Infusions of either 2M NaCl or 0.7M mannitol had no effect on the rats’ daily need-free sodium intake. In addition to changes in sodium appetite, parallel changes in the expression of hypothalamic angiotensin II receptor binding in this brain area was also examined. Together, these data show that direct manipulation of CSF sodium concentration during sodium depletion affects the expression of sodium intake in the rat and that the mechanism by which modification of CSF sodium affects sodium intake may be through its effects on the brain angiotensin II system which then can act as a synergist with aldosterone to elicit sodium appetite in the rat. (Supported by MH43787)

S13.10

ANTHOCYANIN PATHWAYS INVOLVED IN SODIUM APPETITE IN THE RAT. ANATOMICAL CONSIDERATIONS. G.J. Alheid,1 J. Schoulin,2 and A.R. Epstein.1 1Univ. of Wash. Health Sci. Ctr., Charlottesville, VA 22908, and Depts. of Biol. and Anat. Univ. Penn., Phila. PA 19104. (Accepted) It is known that destruction of the stria terminals does not interfere with sodium appetite while large knife cuts in the ventral amygdalar association pathways do. In this study, we are reexamining the role of the ventral pathways with respect to DOCA and ACTH induced sodium appetite by placing small knife cuts in the trajectory of amygdala-fugal and -petal pathways. In addition, behavioral testing is followed by the intravenous injection of fluorescent neuronal tracers for selected animals, in order to provide detailed information about the particular pathways spared by our knife cuts. We observed that small cuts medial to the central amygdaloid nucleus result in a decrease in sodium appetite after fluid volume depletion or repeated Captopril injection, but normal responding to DOCA injections. Surprisingly, in all animals so far examined (n=8), these lesions did not block the projection of the central nucleus to the brainstem, since relatively complete retrograde labeling of the nucleus was possible in every case. Subsequently, we have also injected anterograde tracers into the central nucleus of knife-cut animals with the preliminary results (n=2) suggesting that these cuts might interrupt associative connections with the forebrain, and possibly central amygdalar hypothalamic tracts. Supp. NIMH #017743 (GPA) and NIH #1R03 NS1-2387 (ANE and JS).

S13.11

SALT-TASTE RESPONSES OF FOREBRAIN NEURONS, WHICH ALSO RESPOND TO IONTOPHORETIC APPLICATION OF ANGII, IN AWAKE RATS. S. Nicolaidis,1 M-C. Moussallem and S.N. Thompson. CNRS URA 437, Neuroendocrinology Group, Collège de France, 11 pl. Marcelin Berthelot, 75231 Paris CEDEX 05, France. Perfusion with deoxytocicocctosterone acetate (DOCA) for 3 days sensitizes the rat anterior (thalamus to a subsequent intracranial-ventricular injection of a below threshold dose of angiotensin (ATII) such that a strong appetite for sodium is generated. We have investigated electrophysiological effects of neurons in the septum and preoptic area of anesthetized application (i.c.) of ATII of giving salt solutions to awake rats before and after pretreatment with DOCA. Male wistar rats were anesthetized with ketamine (0.3 mg/kg body weight) and a specially adapted stereotaxic probe fixed to the front of the thalamus. This probe subsequently permits restraint of the rat in a stereotaxic apparatus while allowing the animal to move its body and limbs and to drink water or solutions of salt or glucose. A 7 barreled microiontophoretic electrode sealed to an extracellular recording electrode was then advanced through the cortex into the septum and medial preoptic regions where unit activity was recorded at the same time as different solutions were offered to drink. In the medial septal and medial preoptic areas, but not in other areas, neurons were modulated differentially to salt and water. Some of these salt vs water taste receptors were also modulated by angiotensin II (AngII) or other sodium ions. This is consistent with the hypothesis that these neurons, which can be activated by doxocorticosterone acetate (DOCA), also respond to angiotensin II. (Supported by NE 43787)

S13.12

THE EFFECT OF MINERALOCORTICOID (MC) TREATMENT ON SUBSEQUENT INJECTED SODIUM APPETITE. T.M. Richardson, and N.E. Rowland. Dept. of Psych. U. of FL, Gainesville. Previous studies have shown that rats acutely depleted of sodium drink more NaCl on the second depletion than the first. It has been proposed that the high levels of hormones of sodium depletion (e.g. MC and angiotensin II (AngII)) act synergistically to sensitize brain mechanisms for sodium appetite. In order to assess the generality of this behavioral sensitization as well as the relation of the two hormonal mechanisms, two groups of female Sprague-Dawley rats were treated with 1 week with either an MC (DOCA) or the Ang II enalapril (which is thought to increase brain Ang II activity). Both groups showed the expected salt appetite as measured by 24 hour intakes of 0.2M NaCl and distilled water. After a 1 week rest, a second 1 week period of treatment was performed, with half of the rats receiving the same treatment, and the other half receiving the other (e.g. DOCA,DOCA-enalapril, enalapril-enalapril, enalapril-DOCA). After a further 1 week rest, the rats were switched from DOCA to enalapril showed a paradoxical decrease in salt appetite compared with rats receiving enalapril without prior DOCA. Rats with prior DOCA treatment did not show a change across the phases. These results support the hypothesis that MC and AngII interact to further enhance sodium appetite. (Supported by NS 49945)

S13.13

FOURTH VENTRICLE MICROINJECTION OF DIAZEPAM ENHANCE HEDONIC REACTIONS TO TASTE. S. Petrat* and R.C. Berridge. Dept. of Psychology, University of Michigan, Ann Arbor, MI 48109. Benzodiazepine administration (e.g. 5mg/kg diazepam) enhances hedonic reactions to taste stimuli in rats (e.g. sucrose preferences; Treit & Berridge, 1990). Benzodiazepines also enhance hedonic taste reactivity in decerebrate rats, which suggests that mechanisms within the brainstem mediate this effect (Berridge, 1989). To test this hypothesis in normal animals, we compared the effects on hedonic taste reactivity of intraventricular diazepam injections into the 3rd or 4th ventricles. Twenty rats were implanted with 3rd and/or 4th ventricle cannulae and with oral cannulae. Diazepam (0.5, 15, 25, 40, 50, 75, and 100 ng) was injected into either the 3rd ventricle or the 4th ventricle seven minutes before the rats were tested for taste reactivity to 0.3M sucrose (1ml/min). Diazepam was more effective at enhancing hedonic responses when injected into the 4th ventricle than when injected at the same dose into the 3rd ventricle low dose diazepam doses (40ug) enhanced hedonic taste responses only when injected into the 4th ventricle and produced no hedonic enhancement when injected into the 3rd ventricle. Higher doses (50, 75ug) were effective in both ventricles. These results support the conclusion that benzdiazepine receptors in the brainstem mediate this enhancement of hedonic reactions to taste.

S13.14

NEONATAL CHORDA TYPANI TRANSECTION ALTERS ADULT PREFERENCE FOR NH4Cl IN THE RAT. S.J. Sollars & L. Bernstein. Dept. of Psychology, University of Washington, Seattle, WA 98195. The chorda tympani nerve (CT) is considered the main gustatory pathway for NaCl taste stimuli in the adult rat. However, surgical transection of the CT does not affect preference for salts. The current study examined the effect of transection of CT in 10-day-old rats on adult preference for NaCl. A randomization approach was based on any evidence that primary gustatory nerves play an inductive role in taste bud development with the sensitive period for this effect spanning the first 10 days postnatal in the rat. Ten-day-old Wistar rats were given bilateral transection of the CT (CTX; N=13) or sham operations (SHAM; N=12). When 60-days-of-age the animals had two-bottle access to salt solutions (NaCl, NH4Cl, KCl, CaCl2) and water. CTX animals displayed a significantly higher preference for NH4Cl at all concentrations tested (0.5M, 1M, 1.5M, 2M) while their preference for other salts was not consistently altered. Following the series of two-bottle tests, animals were tested for generalization of conditioned taste aversions (CTA) to salts. LiCl was used to condition a significant CTA to 1.5M NaCl. Tests for generalization were performed by giving one-bottle access to NH4Cl or KCl and a two-bottle test with NH4Cl and NaCl. CTX and SHAM animals showed similar patterns of CTA generalization and the two-bottle test revealed that CTX animals were clearly able to discriminate between NaCl and NH4Cl. The results suggest that neonatal transection of the CT significantly enhances adult preference for NH4Cl and that this effect is not due to the animals’ inability to distinguish between NH4Cl and NaCl.

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Aluminum potassium sulphate (AlKSO₄ 3H₂O) was tested in three experiments, for its ability to elicit sensations of oral astragency. In the first experiment, the perceived astragency of alum (10 g/l) was compared to water on a nongustatory surface by rubbing a cotton roll saturated with stimulus between the gum and upper lip. Astrigent sensations were rated on a visual analog scale that ranged from no sensation to extremely intense. Alum elicited strong astrigent sensations when the lip was moved laterally against the gum to increase friction. In the second experiment, subjects were asked to identify which of two solutions elicited a sensation of astragency in a forced-choice, two-alternative paradigm by simply dipping the anterior of the tongue into two cups: one containing alum, the other a mixture of sucrose, citric acid, and caffeine that approximated the taste elicited by alum. Subjects were asked to identify which of two solutions elicited a sensation of astragency in a forced-choice, two-alternative paradigm by simply dipping the anterior of the tongue into two cups: one containing alum, the other a mixture of sucrose, citric acid, and caffeine that approximated the taste elicited by alum. Subjects were asked to identify which of two solutions elicited a sensation of astragency in a forced-choice, two-alternative paradigm by simply dipping the anterior of the tongue into two cups: one containing alum, the other a mixture of sucrose, citric acid, and caffeine that approximated the taste elicited by alum.

This research was supported by a grant to B.G.G. DC 00249 and training grant DC 00014-13.

INGESTIVE BEHAVIOR: NUTRIENTS, SEROTONIN AND INSULIN


In dietary selection studies, obese Zucker rats choose a greater proportion of their diet as fats than do lean rats. To investigate the contribution of the osmoregulatory function of fats for the control of fat ingestion in these rats, we measured the sham fed intake of several dilutions of corn oil in one bottle intake tests and two-bottle preference tests.

Male Zucker rats (n = 11 obese, n = 7 lean) were fitted with gastric cannulas for sham feeding. In 1-bottle sham feeding intake tests, eight concentrations of corn oil, decreasing in concentration by half and ranging from 100% to 0.75%, were offered to rats in descending order. At the completion of this series of tests, 2-bottle preference tests were performed on two consecutive days comparing preference for 100% and 75% corn oil. There were no clear differences in 2-bottle preference for 100% corn oil, although intake was an average of 50% of the sham fed intake.

Supported by NIHDK08757 and the International Life Sciences Institute (IG).

514.3 INTESTINAL CAPSAICIN ATTENUATES OLEATE-INDUCED SUPPRESSION OF FOOD INTAKE WITHOUT CAUSING ANATOMICALLY DETECTABLE NEURAL DAMAGE OR IMPAIRED OLEATE ABSORPTION. C.S. Tatum and R.C. Ritter.* Dept. of V.C.A.P.P. and Pharmacology/Toxicology Graduate Program, Washington State University, Pullman, WA 99164.

We have previously reported that intraintestinal infusion of capsaicin (5mg/rat) attenuates suppression of sham feeding by intraintestinal oleate infused 24h post-capsaicin (Soc. Neurosci. Abstr. 17:542, 1991). The attenuation is transduced and induced suppression is reestablished by 48h post-capsaicin. To determine whether capsaicin's desensitization of oleate-induced suppression was due to destruction of vagal sensory neurons, we examined the visceral hindbrain for evidence of neural degeneration following intestinal capsaicin infusion. Intestinal capsaicin (5mg) produced no histochemical evidence of sensory neuron degeneration in the dorsal hindbrain. In contrast, intraintestinal injection of capsaicin, 5mg/rat or 50mg/kg, produced degeneration of terminals and fibers in the nucleus of the solitary tract and the spinal trigeminal nucleus. To assess the possibility that intestinal capsaicin damages enteric nerve fibers, we examined substance P-like immunoreactivity (SLI) in whole mounts of intestinal myenteric and submucous plexes. Intestinal capsaicin caused no apparent loss of SLI in either plexus one hour after intraintestinal capsaicin infusion. Finally, we measured intestinal absorption of 1C oleate in vehicle-control and capsaicin-infused rats. Preliminary results indicate that intraintestinal capsaicin does not alter intestinal absorption.

We conclude that capsaicin attenuates oleate-induced suppression of sham feeding by a mechanism other than vagal sensory neuron destruction, substance P depletion or impairment of intestinal absorption. Supported by NIH NS20561 to R.C.R.

514.4 DOSE-DEPENDENT EFFECTS OF DUODENAL AND ILEAL INFUSION OF GLUCOSE ANDOLEIC ACID ON MEAL PATTERNS IN RATS. T.A. Woltman and R.D. Reidelberger. Veterans Administration Medical Center and Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68105.

The mechanisms mediating the inhibition of feeding by luminal nutrients in the proximal and distal small intestine are not clearly understood. In the present study we determined the dose-dependent effects of duodenal and distal ileal infusion of glucose and oleic acid on meal patterns in ad libitum feeding rats. Animals (n=8-12) with chronic cannulas in both the duodenum and ileum (14 cm proximal to the cecum), received a 2-h infusion (0.13 ml/min) of glucose (0.01, 0.02, 0.04, 0.08, and 0.16 kcal/ml) or oleic acid (0.003, 0.017, and 0.085 kcal/ml) at the start of the dark period, and meal patterns were monitored for 19 h. Results: Three-hour cumulative intake was inhibited dose-dependently by ileal as well as duodenal infusion of both glucose and oleic acid. Ileal glucose was more inhibitory than duodenal glucose, while duodenal fat was more inhibitory than ileal fat. Duodenal glucose and fat inhibited feeding by decreasing meal frequency; ileal fat decreased only meal size, while ileal glucose reduced both meal size and frequency. Conclusion: It appears that distinctly different mechanisms mediate the inhibitory effects of duodenal and distal ileal glucose and fat on food intake.
514.5
POSTMEAL FATTY ACID OXIDATION DEPENDS ON MEAL COMPOSITION. D.M. Spry*, W. Langhans, R.P. Reeves, C. Wenk.
Institute of Animal Sciences, Swiss Federal Institute of Technology, 8092 Zürich, Switzerland.
The influence of macronutrient content of a meal on fatty acid oxidation was investigated in 13 Caucasian males after consumption of a high fat (HF) breakfast (33% CHO, 52% Fat, 15% Protein, containing equal short- and medium-chain fatty acids, and after a high carbohydrate (HC) breakfast (78% CHO, 6% Fat, 15% Protein). Respiratory quotient (RQ) and plasma beta-hydroxybutyrate (BHβ) were measured during the three hours following the meal as indicators of whole body substrate oxidation and hepatic fatty acid oxidation, respectively. Plasma insulin, glucose, lactate, free fatty acids (FFA), and triglycerides were also determined. RQ was significantly lower and plasma BHβ higher after the HF meal than after the HC meal, implying that more fat is burned in general and in the liver after a HF meal. As expected, plasma FFA and triglycerides were higher following the HF meal, and insulin and lactate were higher after the HC meal. In sum, considerable fat oxidation occurred in response to a single high fat meal, without prior adaptation to a high fat diet. The short- and medium-chain fatty acid content of the HF meal may be the primary contributor to the observed increase in plasma BHβ, which reflects elevated hepatic fatty acid oxidation. This is interesting in relation to previous studies which link changes in hepatic fatty acid oxidation to the control of food intake.

514.7
ANORECTIC EFFECTS OF A NOVEL DOPAMINE- FATTY ACID BIOCONJUGATE. G.W. Hesset and V.E. Shaboua.
Ralph Lowell Laboratories, McLean Hospital, Harvard Medical School, Belmont, MA 02178.
A novel pharmacological agent consisting of a fatty acid amide linked to dopamine has been synthesized. This novel compound has been found to have anorectic effects. It inhibits food consumption by fasted mice in a dose dependent manner with an ED50 of about 15 μmol/kg. The anorectic effects of this compound are antagonized by sulpiride, but not by domperidone, indicating that central nervous system dopaminergic receptors are involved. Chronic administration of this compound produces little or no tolerance. During daily administration of the compound for 21 days no loss of efficacy in appetite suppression in fasted mice was observed. At the end of the 21 day period, food consumption returned to the baseline level within 24 hours. Finally, the compound does not induce hyperactivity, stereotypy or catalepsy even at doses up to 100-fold above the ED50. This novel compound is an effective appetite suppressant which appears to lack the excitatory effects on behavior often associated with dopaminergic anorectics.

514.8
INFLUENCE OF INTRAVENOUS NUTRIENTS ON FOOD INTAKE AND ENERGY EXPENDITURE. E.K. Walls* & H.S. Koopmans.
Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.
Metabolic theorists propose that satiety is produced by relative increases in the rate of oxidation of fuels. To determine if intravenous (iv) nutrient-induced satiety is dependent upon increased fuel oxidation, daily food intake (17 hr/day) and 24 hr energy expenditure (EE) were measured in rats during iv nutrient infusions in rats. Infusion of 52 kcal of glucose and amino acids (GAA) for 4 days immediately reduced food intake by 38.4% or 74% of the nutrient calories infused. EE on day 1 of GAA (57.8 ± 1.2 kcal) was not premeditatedly administered on day 1 of GAA (58.1 ± 1.3 kcal) but increased to 61.0 ± 1.3 kcal on day 4 (p < .05) whereas EE values increased from 938 during saline to 1,076 and 1,086 (p < .001). Infusion of 40 kcal of lipid for 6 days reduced food intake by 21.9 kcal or 55% of the lipid calories infused. EE on day 1 and 6 of the lipid infusion (62.7 ± 2.2 and 62.6 ± 2.4 kcal) were not greater than during saline control (62.6 ± 2.1 kcal), whereas EE values were reduced from 904.8 and 911.8 on day 1 and 6 (p < .001). While the shifts in EE indicate enhanced fat utilization in the lipid condition and increased carbohydrate utilization in the GAA condition, the fact that no changes in EE accompany large changes in food intake suggests that signals for iv nutrient-induced satiety are not generated by large increases in fuel oxidation.

514.9
PERIPHERAL CHRONIC CLONIDINE DOES NOT AFFECT SUSCEPTIBILITY TO ACTIVITY-BASED ANOREXIA. T.S. Rieg*,
J. Cho*, and P.F. Arach. Department of Anatomy & Neurobiology, Eastern Virginia Medical School, Norfolk, VA 23060; Medical Research Service, VA Medical Center, Hampton, VA 23318.
Activity-based anorexia (ABA) is an animal model of anorexia nervosa (AN) with two characteristics of the disorder, decreased food intake and increased activity. We have previously shown that chronic stimulation of the paraventricular hypothalamus with the noradrenergic agonist clonidine exacerbates ABA rather than ameliorates it as predicted. This study determined if peripheral chronic administration of clonidine would affect ABA. Rats were implanted subcutaneously with self-contained micropumps infusing saline, 30, or 300 μg/kg/day. Following postsurgical recovery, all animals were exposed to ABA (1.5 hrs/day/ad lib food; 22.5 hrs/day/ad lib wheel access) for 14 days with exposure days to a 25% body weight-loss criterion. Results showed that clonidine did not affect susceptibility or food intake, but substantially increased wheel activity in a dose related fashion. These findings are perplexing in that susceptibility to ABA was not increased although the high dose animals were running more. This may be due to an inhibition of sympathetically mediated energy expenditure by clonidine in ABA.
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In previous work it has been shown that adult male offspring of rats that have been injected with Glu/kg during the first two weeks of gestation (Jones & Daynes, 1990) or undereuthrained to 50% of baseline intake during the first two weeks of gestation only (Jones & Friedman, 1982) develop significant obesity commencing at about 50 days of age. The present experiment examined the question of whether rats with these two forms of obesity display abnormalities in the release of the brain known to influence feeding and body weight. Twenty-one gauge stainless steel guide tubes, were chronically implanted using standard stereotaxic procedures. One week later 1 pmol microdialysis probes were lowered into the medial hypothalamus. Diacylate collection began 12 hours later. Diacylates collected from both male and female animals in the two experimental conditions contained significantly higher Norepinephrine (p<.05) than did controls. It would appear that in addition to sharing a similar time course of onset and a sex dependent expression of obesity (only males become obese), both of these models of obesity are also characterized by elevated medial hypothalamic norepinephrine. Since expression of this obesity is sex dependent, we investigated whether sex steroids might be mediating the effect. Gonadal weights were measured and plasma estrogen and testosterone levels were measured. These data will be presented.


Studies have shown that carbohydrate is the preferred macronutrient in the first half of the nocturnal feeding cycle. It has been proposed that serotonin (5-HT) is involved in switching off this initial carbohydrate meal, acting through hypothalamic 5-HT receptor mechanisms that control satiety. To provide a further test of this hypothesis, we have examined, in male albino rats, the impact of PVN injections of 5-HT (2.5 mmol) and i.p. injections of the 5-HT antagonist, metelegorine (MTG, 10 mg/kg), administered at dark onset, on the microstructure of feeding over the 12 hr nocturnal cycle. In the PVN, 5-HT produced a selective decrease in carbohydrate intake. This effect, while still evident 8 hrs after injection, was exhibited primarily during the first meal of the feeding cycle. During this meal, there occurred a selective decrease in size, percent composition, feeding time and feeding rate for carbohydrate, as well as an increase in the satiety ratio (post-meal interval:meal size) for this nutrient. In contrast, i.p. MTG injection produced opposite effects, namely, a selective stimulation of carbohydrate ingestion, an increase in the rate of carbohydrate feeding, and a decrease in the satiety ratio for this nutrient. This effect was followed 3 hrs later by a potentiation of protein, but not fat, intake. These results indicate that 5-HT may be involved in switching off preference for the carbohydrate diet at the onset of the natural feeding cycle.

514.15 HYPOTHALAMIC 5-HT 2 RECEPTOR SUB-TYPES ARE INVOLVED IN CONTROL OF GLUCOSE RELATED FEEDING. B. Moorjani, P. Lacey*, and M. Pivarnik-Jung. The Rockefeller University, New York, NY 10021 and Osteopathic Med/Hlth Sci., Des Moines, IA.

The sulphonylurea tolbutamide (TOL), a hypoglycemic agent, and the 5-HT 2 agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), cause hypoglycemia. This study explored the mechanism common to these hypoglycemic actions. The objective was to examine the effect of chronic and acute TOL, and glucose (GLU) replacement therapy, on the 5-HT 2 receptor density, in the hypothalamus (MH), amygdala (AL), and subiculum (Sub) of male Sprague-Dawley rats. Rats were given one of the following treatments: a) 5 chronic injections (i.p.) of either propylene glycol (vehicle), TOL (25mg/kg), or TOL + GLU (20%; b) acute injections (i.p.) of vehicle or TOL (50 mg/kg post-injection), their brains were removed, and the MH and LH were dissected. The radioligand binding technique was used to estimate the binding of [3H]8-OH-DPAT (2 nM) to 5-HT 2 receptor sites (in the presence or absence of 10uM 5-HT). Trunk blood was collected for estimation of plasma glucose. The results show: 1) TOL induced a significant decrease in GLU levels in both chronic (57.8%) and acute (-52%) states; 2) TOL produced an increase in 5-HT 2 receptor density in the MH under both chronic (314%; p<0.01) and acute (132%; p<0.01) conditions; 3) GLU replacement caused a partial restoration of receptor density (-45% from the TOL treated rats). These findings suggest that the medial hypothalamic 5-HT 2 receptor sub-types may interact with blood glucose in the control of feeding behavior, and TOL may induce hypoglycemia via activation of 5-HT 2 receptors.

514.16 REDUCTION IN DARK ONSET FEEDING AND CARBOHYDRATE INTAKE IN GENETICALLY OBSE (ob/ob) AND LEAN (1/2) MICE INJECTED WITH 5-HYDROXYTRYPTAMINE P.J. Curr**, L.M. Wilson*, R. Clark* Institute of Psychiatry, University of Toronto, Toronto ON M5T 1R5 and *Department of Psychology, University of Manitoba, Winnipeg MB R3T 2N2 Canada.

The genetically obese (ob/ob) mouse exhibits multiple disturbances in neural systems putatively involved in the control of feeding, including altered levels of brain 5-hydroxytryptamine (5-HT) and reduced 5-HT metabolism. 5-HT has also been implicated as the control of carbohydrate (carbohydrate, protein, and fat) intake in free-feeding obese mice (g=7) and lean controls (g=7). Mice were injected with 5-HT (35-140 nmol) or saline physiological saline, in intraperitoneal doses, immediately prior to dark onset. 5-HT decreased feeding and carbohydrate intake dose-dependently (p<.05), and as a result, tended to decrease the proportion intake of protein and fat. Obese mice, however, showed a reduced sensitivity to the anorectic effect of exogenously administrated 5-HT. Although these results are consistent with a role for serotonin in the control of feeding and carbohydrate intake in mice, the altered sensitivity of the ob/ob to 5-HT treatment may result, in part, from an impaired anorectic control mechanism in this genetic strain. (Supported by NSERC of Canada to LMW and a MIRC predoctoral scholarship to PJCC).
S14.17


Finkel (1980) proposed that the fuel rich environment experienced by a fetus of a gestational diabetic could result in an abnormal insulin system, thereby predisposing to obesity throughout life and more likely to develop gestational diabetes. Critical events in neural and hormonal development during late gestation in humans occur in early postnatal life in the rat. We have shown that overfeeding rats during this critical time produces adult rats that are frankly obese and either unable to carry pregnancies to term or producing significantly smaller pups.

The purpose of this study was to examine the immediate effects of overfeeding on insulin receptor binding in brain and liver. At postnatal day four, rats were assigned to one of four treatment groups: (1) mothered (MR); (2) Gastronomy-fed to weight match MR's (WM); (3) Gastronomy-fed with excess formula (OF). On day 14 the animals were sacrificed and cerebral cortices and livers were assayed for insulin receptor binding.

At sacrifice of animals were significantly larger than MR's or WM's. Insulin binding was greatest for MR's and least for OF's. A similar but not significant trend was seen in cortical binding.

Hypersensitivity is seen in babies of gestational diabetics. Our data suggest that overfeeding during the second postnatal week may induce hypersensitivity leading to a decrease in insulin binding in liver; (2) formula feeding in itself may cause a lesser degree of hypersensitivity.

S14.18

DISCRIMINATION OF INSULIN-PRODUCED HYPOGLYCEMIA
P.M. DUNCAN* and W. LICHTY, Psychology Dept.
Old Dominion University, Norfolk, VA 23508.

Eight rats were trained to discriminate the normal state of euglycemia from the hypoglycemia produced by injection of 6 units/kg insulin.

A "drug discrimination" procedure was used with two-lever Skinner boxes and a food-motivated operant schedule. In each group no-reinforcement was followed by 6 min during which either left, or right lever presses were reinforced on a VI 10-sec schedule. Insulin or water injection 25 min prior to the operant session determined whether left, or right lever presses were reinforced. During discriminating training, reliable discrimination of the insulin-produced cue developed, with a mean of 73% responses on the "insulin lever" after insulin injection, and only 18% after water injection. After insulin, or water injection mean blood glucose levels dropped to 75%, or rose to 127% pre-injection values. When injected with 800 mg/kg ethanol, most rats chose the non-insulin ("euglycemic") lever. These results show that insulin produces a specific interoceptive cue, presumably involving hypoglycemia.

S14.19

PARAVENTRICULAR HYPOTHALAMIC INJECTION OF 7-Chlorokynurenic Acid (7CK) STIMULATES FEEDING IN SATIATED RATS. *T.L. Norrell and E. Bastock, Psychology Dept., Queens College, CUNY, Flushing, NY 11367.

7CK (3ug & 6ug) and 2-aminoo-5-phosphonovaleric acid (AP5, 5ug & 10ug) were injected into the following hypothalamic sites: anterior h(4th), lateral h(4th) ventromedial h(5th), dorsomedial h(5th), and the paraventricular nucleus (PVN) of satiated rats and 1 hour consumption of pellet food was measured. Rats injected in the third ventricle anterior or posterior to the PVN were also given 7CK. AP5 failed to increase feeding at any site at the doses tested. 7CK data

Area(s) + LIH(4th) AH(5th) VMN(6th) DMN(6th) PVN(9th)

7CK 0.44±0.2 0.6±0.2 0.77±0.2 0.6±0.2 0.8±0.2
AP5 0.38±0.2 0.38±0.2 0.77±0.2 0.77±0.2 0.77±0.2

Area(s) VM(11th)Anterior -> VM(11th)Posterior -> VM(11th)

7CK 0.86±0.2 0.46±0.2 0.13±0.2
AP5 0.86±0.2 0.38±0.2 0.13±0.2

Insulin in hypothalamic sps. following vehicle was 0.39±0.09

PVN animals also received a test dose of scopolamine 40 ug which produced a feeding response of 4.11±5.4 gms.

S15.1

THE EFFECTS OF ALCOHOL ON INHIBITORY MECHANISMS IN RAT HIPPOCAMPAL CA1 NEURONS IN VITRO. J.R. Criado* and R Thies. Dept. Psychiatry, University of Washington Physiology and Pharmacology, Univ. of Oita HSC, Oita City, Oita, Japan.

Alcohol typically inhibits neurons, which may be due to potentiation of inhibitory GABAergic systems. Alcohol also may excite neurons, which could be due to either direct facilitation or disinhibition of tonically active GABAergic interneurons. Potentiation of the effects of GABA by alcohol has been shown in biochemical and behavioral data but not in electrophysiological studies. This study examined the acute effects of various doses of i.v. alcohol on GABAergic inhibitory mechanisms in hippocampal CA1 neurons.

The paired pulse paradigm was used to test the effects of alcohol on the activity of local inhibitory circuits in the CA1 region. Local groups of neurons (recording population spikes) were activated by paired pulses from the same source (orthodromic-orthodromic) or from two different sources (antidromic-orthodromic). Local inhibitory circuits were characterized by mapping the period of inhibition with GABAergic agonists and antagonists released from a multibarrel pipette.

Recurrent inhibition was unaffected by alcohol, but alcohol prolonged the inhibition in rats treated with a current injection. Consequently, alcohol probably potentiates feedforward inhibition, possibly by affecting post- or pre-synaptic GABA<sub>B</sub> receptors (Supported by the Department of Psychiatry and Sigma Xi).

S15.2


The effects of chronic alcohol exposure and withdrawal on the GABAergic and non-GABAergic synapses of the dentate molecular layer (DML) have been studied in the alcohol-sensitive SJL and alcohol-insensitive DBA mice, respectively (28 animals total). Mice were fed for 4 mo with either a control isocaloric liquid diet or with a 27% isocaloric liquid diet containing ethanol (23.5% ethanol derived calories). Half of the ethanol-treated mice were withdrawn from the diet for 1 mo. All animals were treated for GABA immunoelectron microscopy in the potentiometry procedure. Out of the total synaptic population in the middle and distal thirds of the DML, 91% and 87% of synapses are on dendritic spines and 9% and 13% are on dendritic shafts, respectively. Within the population of asynaptic and axosynaptic synapses, the GABAergic contacts represent 60% and 3%, respectively. There are more synapses on dendrites in the distal than in the middle third, while on spines, the contacts are equally distributed across both thirds. During ethanol exposure, there was a significant loss (24%) of axosynaptic synapses in the distal third of the DML. This change was transient and returned to control values during withdrawal. However, in the GABAergic synapses there was a progressive loss of axosynaptic contacts which peaked during the withdrawal period (41% and 37% in the middle and distal thirds, respectively). Given that in the DML the major synaptic input is on dendritic spines, the ethanol-induced loss of the excitatory axosynaptic synapses could affect the activity of dentate granule cells. The loss of the GABAergic inhibition could be secondary to the reduced excitation. The sequence in which these changes occur supports such a conclusion. Functional validation of these synaptic changes remains to be established in the dentate fascia. However, in chronic ethanol preparations, the CA1 pyramids display a reduced capacity to induce long-term potentiation which could result from a decreased excitatory synaptic input (Tremel and Hunter, Alcoholism, 15:35A, 1991). Supported by AA06966.
5.5.3 ETHANOL DIFFERENTIALLY MODULATES SYMPATHETICALLY EVOKED GABA, RECEPTOR-MEDIATED RESPONSES FROM HIPPOCAMPAL, CORTICAL AND SEPTAL NEURONS IN RAT BRAIN SLICES. B.L. Schild, W.R. Proctor and T.V. Dunnwald, University of Colorado Health Sciences Center, and Veterans Admin. Medical Research Services, Denver, CO.

Previous electrophysiological studies have reported variable results concerning the effect of ethanol on GABA-receptor mediated responses in the CNS. The present study was designed to determine whether ethanol modulation of GABA response is brain region dependent, and to identify factors that might regulate ethanol sensitivity. We examined the effects of ethanol on sympathetically evoked GABA-IPSCs, which were studied with whole-cell voltage clamp recordings from neurons in three brain regions (hippocampus, medial cortex, and septum) that have been reported to differ in their degree of ethanol modulation. Bicuculline-sensitive IPSCs elicited by local stimulation were isolated by pretreatment with the glutamate specific antagonist kynurenic acid (KYN, 100 μM). Ethanol (0-160 mM) did not significantly modulate evoked GABA, IPSCs in CA1 pyramidal neurons. In contrast, ethanol potentiated these responses in cortical neurons (layer V), and in medial and lateral septal neurons. However, even in those areas, there were neurons whose IPSCs were unaffected by ethanol. These results suggest that ethanol modulates responses to endogenous GABA released during synaptic activation, that there are overall differences in GABA receptors from various brain regions, and that there may be additional factors (such as heterogeneous subpopulations and local endogenous neurotransmitters) that affect ethanol sensitivity within certain brain regions.

Supported by grant AA03527 and the V.A. Medical Research Services.

5.5.5 ETHANOL ENHANCEMENT OF GABA-MEDIATED CHLORIDE CURRENTS IN ISOLATED CEREBELLAR PURKINJE CELLS IS EXERTED BY AN INCREASE IN BURST DURATION AND STEPS TO MULTIPLE LEVELS OF THE PREDOMINANT SINGLE CHANNEL CONDUCTANCE. H.P. Fahrenkrug and G.J. Woodard. CUBU-De Univ. de Colinas, CO., 28015 Mexico Dept. of Pharmaceutical, and Pharmacol., Bowman Gray School of Medicine, Wake Forest Univ., Winston-Salem, NC 27106.

This work was undertaken in order to characterize the mechanism of enhancement of the actions of ethanol on GABA-mediated chloride currents (GABA) in dissociated Purkinje cells from 1-8 days old rats. In the whole cell configuration, and in pipette (1 pH 7, 17 sec) of ethanol (10 to 50 mM) enhanced up to 127% the current evoked by GABA (10 μM) at 10-100 μM (10 sec). Repetitive (every 30 sec) ethanol pulses or continuous bath perfusion induced a gradual potentiation (close dependent) up to 300% above control after 20 min of unaltered (10-50 mM) application. In cell-attached (patch) and inside-out configurations, ethanol (10-50 μM) induced initially an increase in GABA-evoked channel open time (GABA concentration in pipette: 0.5 μM), followed by steps to multiple levels of the predominant single channel conductance. We conclude that ethanol enhancement of GABA takes place by direct interaction of ethanol with the GABA receptor, which increases the frequency of occurrence of channel opening and/or a change in the gating properties, as well as by increasing recruitment of additional channels. Part of this study supported by DGCICA-SEP, CONACYT (MPP); NIHAAI 03911-11 (SDN).


In previous electrophysiological studies, we have shown that ethanol potentiates GABA-induced depressions of cerebellar Purkinje neurons when these responses were simultaneously augmented (modulated) by a β-adrenergic agonist, such as norepinephrine or isoproterenol (ISO). Age-related differences have deficits in the postynaptic function of β-adrenergic mechanisms in the cerebellum and, thus, might be expected to be less sensitive to the ethanol-induced potentiation of the effects of ethanol in this brain area. In the present study, we found electrophysiological evidence which suggests that not only does the efficacy of locally-applied ISO for potentiating cerebellar GABA responses decrease with aging in F344 rats, but the potency of the effects in this brain area. In the present study, we found electrophysiological evidence which suggests that not only does the efficacy of locally-applied ISO for potentiating cerebellar GABA responses decrease with aging in F344 rats, but the potency of the effects in this brain area.

In the age groups of 6 months old and 2 months old were used. The effects of ethanol are significantly decreased in the 6 months old age group. These findings further support the importance of the ethanol in different brain areas.


Ethanol is reported to alter the action of excitant amino acids (EAA) and GABA in several brain sites. The brainstem reticular formation (BRF) plays an important role in propagation of generalized seizures, including audiogenic seizures (AGS). Previous studies indicate that microinjection of an EAA antagonist, APT, at the posterior BRF blocks AGS in the GEPR. Effects of microinjection of GABA agonists into the R of the GEPR have not been reported. In the present study, Sprague Dawley rats were implantated with guide canulae bilaterally over the pontine RF (nucleus gigantocellularis) stereotaxically. Ethanol was administered intragastrically (9-15 g/kg/day). At the end of day 4, ethanol was withdrawn. Animals exhibiting AGS at 10h after ethanol withdrawal (ETX) received infusions of vehicle (phosphate buffer or dimethylsulfoxide) into the RF without effect. Vehicle infusion was followed by infusion of an AGS antagonist (CPP), a non-GABA antagonist (CNQX) or a GABA-A agonist (THP). CPP (10mmol/side) and CNQX (10mmol/side) suppressed AGS during ETX. THP (10mmol/side) also blocked AGS during ETX. Thus, these results indicate that the ethanolic effects of enhanced EAA and reduced GABA neurotransmission in the RF in AGS susceptibility during ETX. These findings further support the importance of the RF in several forms of AGS and audiogenic-like seizures. (Supported by NIAAA AA06931).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
$15.9$ **AFFECT OF ALCOHOL DEPENDENCE AND WITHDRAWAL ON mRNA FOR GABA$_A$ RECEPTOR SUBUNITS AND CCK IN MICE.** D. Reuss, P. J. Peterson, M. Field, J. A. Post and J. Higgins*, Parks-Davis Neuroscience Research Center, Addenbrookes Hospital Site, Hills Rd., Cambridge CB2 0QB.

A period of chronic ethanol treatment followed by withdrawal from the drug, resulted in pronounced neuronal and chromatographic changes in GABA$_A$ receptor activity. Recently we have shown that the novel anxiolytic, CI-988, a selective CCK$_4$ receptor antagonist, inhibits withdrawal induced behaviors. The present study examines the effect of ethanol withdrawal throughout a variety of time intervals, on levels of mRNA for CCK and GABA$_A$ receptor, 6n and 7/15R in the brain. Mice were exposed to 8% ethanol, given as a liquid diet, for 17 days, mice were withdrawn for 16 hours and then slowly infused i.v. with N-methyl-DL-aspartate (NMDA) until a clinical seizure was attained. CI-988 (0.1-1.0 mg/kg s.c.) was given at the start of the withdrawal period and 40 minutes prior to testing for spontaneous and NMDA-induced seizures. mRNA for GABA$_A$ receptor subunits and CCK was measured by Northern blot analysis and slot-blot hybridization using digoxigenin labeled probes at the 3' end with [35S]ATP. Ethanol treatment and withdrawal produced a number of changes in the mRNA for GABA$_A$ receptor subunits although mRNA for CCK was unaffected at any phase of the experiment. During withdrawal, the mean convulsive dose (MCD) of NMDA decreased from a control value of 143 ± 8.7 to 72 ± 8 mg/kg. CI-988 dose-dependently antagonized the convulsant actions of NMDA, with a maximum effective dose of 0.1 mg/kg (MCD of NMDA = 112 ± 64 mg/kg), although a consistent response was not seen in all animals. The results suggest that CCK$_4$ receptor activation may play a role in alcohol withdrawal, although this does not appear to involve an increase in CCK mRNA. It is possible that CCK$_4$ receptor activation is the more important function.

$15.11$ **INTERACTION OF TEMPERATURE AND ETHANOL ON S. PREGNAN-3o-OH-20-ONE (3x,5x-P)-INDUCED ALTERATIONS OF GABA-STIMULATED CHLORIDE UPTAKE IN LS AND SS MICE.** PRELIMINARY STUDIES. M. Bejanazz, D.L. Allen*, Dept. of Pharmacological and Toxicological, Univ. So. California, Los Angeles, CA 90033.

Offsetting hypothermia during intoxication increases sensitivity to ethanol-induced loss of righting reflex in SS mice and decreases sensitivity in LS mice. Preliminary studies suggest that toxicogenic differences in the interaction of temperature and ethanol on the GABA$_A$ receptor complex (GBRC). The present study investigated the interactive effects of temperature and ethanol on 3x,5x-P-induced alterations of GABA-stimulated chloride uptake in LS and SS mice using slice preparations. 3x,5x-P (10 µM) produced a significant increase in GABA-stimulated chloride uptake in LS mice and a non-significant increase in SS mice at 30, 34 and 38°C. Ethanol (50 mM) did not significantly alter the effects of 3x,5x-P on the GBRC at 30 and 34°C. At 38°C, ethanol significantly decreased the effects of 3x,5x-P on GABA-stimulated chloride uptake in LS mice and produced a non-significant increase in SS mice. In the absence of 3x,5x-P, ethanol (25-100 mM) produced a significant decrease in GABA-stimulated chloride uptake at 30°C, but not 34 and 38°C in LS mice, and had no effect at any of the tested temperatures in SS mice. Although speculative, these preliminary results suggest that the effects of temperature on behavioral sensitivity to ethanol may involve temperature-induced alterations in the 3x,5x-P and GABA site in LS mice, and the 3x,5x-P site in SS mice (Supported by USF grants AA03972, AA0252, NS25986 and NS24645).

$15.12$ **FAILURE TO "PRECIPITATE" SIGNS OF ETHANOL WITHDRAWAL WITH A GABA ANTAGONIST AND A BENZODIAZEPINE INVERSE AGONIST.** S. Rassnick*, E. Krichman and G. F. Koob, Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

Previous work has shown that spontaneous withdrawal from ethanol (E) and precipitated opiate withdrawal produce a decrease in the locomotion of rats responding for food reinforcement, suggesting that this effect reflects a disrupted motivational state during withdrawal from chronic drug administration. The present study was designed to test whether administration of bicuculline methiodide, a competitive GABA receptor antagonist, or RO 15-4513, a benzodiazepine inverse agonist, would "precipitate" signs of withdrawal during chronic E intoxication. It was hypothesized that an increased sensitivity to the response-disruptive effects of these drugs during E intoxication would provide evidence for a "precipitated" E withdrawal syndrome. Rats were trained to lever-press for food on a fixed ratio-15 schedule of reinforcement, then maintained E dependent for 2 weeks on a liquid diet containing 35% E-derived carbohydrates, or maintained on a control liquid diet. E-dependent rats displayed a decreased sensitivity, rather than an increased sensitivity to the response-disruptive effects of bicuculline methiodide (100 ng/ICV) and RO 15-4513 (3 and 6 mg/kg). The inability of these drugs to "precipitate" E withdrawal is consistent with recent biochemical studies which show that chronic E intoxication produces a down-regulation or uncoupling of activity at the GABA/benzodiazepine receptor complex (Supported in part by NIAAA grants: AA 05297, AA 06420, and The Alcoholic Beverage Medical Research Foundation).

**DRUGS OF ABUSE: COCAINE'S INTERACTION WITH NON-DOPAMINE SYSTEMS**

$16.1$ **BLOCKADE OF SENSITIZING EFFECTS OF AMPHETAMINE PREEXPOSURE ON COCAINE SELF-ADMINISTRATION BY THE NMBA ANTAGONIST MK-481.** R. Hajirasouliha, R. Valdez, D.A. Highfill, T.A. Schafer and S.J. Grant*, Dept. of Psychology, Texas A&M Univ., College Station, TX 77843.

Rats preexposed with amphetamine acquired cocaine self-administration (0.125, 0.25 or 0.5 mg/kg) at a faster rate than saline preexposed rats. This suggests that repeated exposure to this drug prior to self-administration testing sensitized the rats to the reinforcing effects of cocaine. Co-administration of 0.25 mg/kg IP, a non-competitive NMBA antagonist, blocked the ability for chronic exposure to amphetamine to sensitized rats to cocaine; these rats' rate of acquisition of cocaine self-administration did not differ from saline preexposed controls. Thus, glutamate system and the NMBA receptor in particular may play a critical role in the sensitization produced by intermittent exposures to amphetamine. The NMBA antagonist also affected the rate of the activating effect of an acute injection of amphetamine. A suppression of the activating effects during the first 30 min post-amphetamine and an enhancement of the activating effects during the last 30 min post-amphetamine were produced by MK-481. These data suggest that the behavioral expression of an acute exposure as well as chronic administration of this drug (ie., sensitization) may rely on an intact NMBA receptor system. Recent studies on MK-481 administration rats, MK-481 also shifted the dose response curve for cocaine self-administration to the right, suggesting an antagonism of the reinforcing effects of cocaine even in rats that had already unlearned the drug such as in block of the VTA of chloride hydrate anesthetized rats. Standard physiological and anatomical criteria were used to identify DA neurons from the subcaudal ventral tegmental area of the VTA of rats. These studies demonstrate that dopaminergic hypothalamus unique sensitivity to the behavioral response of a DA neuron in the VTA and not the SN. As previously seen in the LC, BUP had a long duration of action and could not be reversed by the opioid antagonists naloxone or naltrexone (up to 10 mg/kg).

These studies demonstrate that amphetamine has a unique property such as the VTA and the LC. Since BUP does not exhibit limited efficacy, partial agonist, mixed, agonist-antagonist properties on the VTA. These data are consistent with the hypothesis that in vivo BUP acts acutely as an agonist. The effects of acute and chronic BUP pre-treatment on morphine actions in the VTA and PAG.

$16.2$ **BUPRENORPHINE IMMUNICS MIMICS MORPHINE ACTIVATION OF DOPAMINE NEURONS, IN MORPHINE, S. J. Grant*, G. Scott, D.A. Highfill, T.A. Schafer and S.J. Grant*, Dept. of Psychology and Prog. in Neuroscience, Univ. Delaware, Newark, DE 19716.

Buprenorphine (BUP) is a synthetic opioid proposed as a potential treatment for cocaine craving. It is a partial agonist at the mu-receptor, described as a partial opioid agonist, but little is known of its electrophysiological effects. We previously reported that BUP, like morphine, completely suppressed the spontaneous firing of noradrenergic neurons of the locus coeruleus. We now report that BUP also mimics the morphine-induced activation of dopamine cells.

Extracellular single unit activity was recorded from dopaminergic (DA) neurons in the ventral tegmental area (VTA) of chloral hydrate anesthetized rats. Standard physiological and anatomical criteria were used to identify DA neurons in the VTA (10-40 µm). The I-V relationship of function), i.e., activated DA neurons in the VTA, but not the SN. As previously seen in the LC, BUP had a long duration of action and could not be reversed by the opioid antagonists naloxone or naltrexone (up to 10 mg/kg).

These studies demonstrate that buprenorphine has unique properties. The VTA and the LC. Since BUP does not exhibit limited efficacy, partial agonist, mixed agonist-antagonist properties on neural activity. These data are consistent with the hypothesis that in vivo BUP acts acutely as an agonist. The effects of acute and chronic BUP pre-treatment on morphine actions in the VTA and PAG. Supported by NIMH, the State of Delaware, and ICI Pharma.
516.3

SELECTIVE REGULATION OF MU AND KAPPA OPIOD RECEPTORS FOLLOWING REPEATED EXPOSURE OF GUINEA PIGS TO COCAINE. Y. Itzhak and I. Stein, Dept. of Biochemistry & Molecular Biology, REPSCED Labs, University of Miami School of Medicine, Miami, FL.

The precise neurochemical mechanisms involving cocaine-induced psychological and neurophysiological adaptations are not entirely clear. Since a few studies implied the involvement of the opioid system in the reinforcing effects of cocaine, we sought to investigate the regulation of opioid receptor following repeated exposure to the drug. Guinea pigs were treated with either saline or cocaine (40 mg/kg/day; i.p.) for 7 days, sacrificed 24 h after the last treatment and various brain regions were dissected and prepared for opioid receptor binding. The three subtypes of opioid receptors, mu, delta and kappa, were labeled with [3H]DAGO, [3H]JDPDE and [3H]U50,933, respectively. A significant down-regulation of mu-opioid receptors (40-60% of control [Bmax]) was observed in the frontal cortex, amygdala, hippocampus and thalamus. No change in mu-opioid receptor binding was detected in caudate putamen, substantia nigra, neocortex and hypothalamus.

Delta-opioid receptor binding in the various brain regions of cocaine-treated animals did not differ from control. Kappa-opioid receptor binding was significantly increased (135.5±5% of control) only in the cerebellum, a region that contains primarily kappa-opioid receptors in guinea pig brain. Taken together, these findings indicate that mu and kappa opioid receptor subtypes are differentially affected by cocaine administration and that these distinct alterations in opioid receptors may be associated with the neurochemistry of cocaine-addiction. Supported by NIDA DA05789.

516.5

Behavioral Evidence for Changes in 5-HTγ Receptor Sensitivity During Withdrawal From Continuous or Intermittent Cocaine. JOYNER, C. M, KING, G. R., SUNCAK, K. S., ELWOOD, E. H., JR. Changes in 5-HT neuronal transmission and neurotransmitter receptor function may affect the efficacy of cocaine's effects and tolerance to chronic cocaine administration. Rats were pre-treated with 40 mg/kg/day of cocaine for 14 days by either subcutaneous injections or continuous infusion by osmotic minipumps. The rats were then withdrawn from the pretreatment regime for 7 days and behaviorally assessed. In Experiment 1, rats received 0, 0.5, 1.0, or 2.0 mg/kg i.p. injections of NANN-190, 1-(2-methoxyphenyl)indoleproline, a putative 5-HTγ receptor agonist. In Experiment 2, the rats received the same doses of NANN-190 in combination with a 15 mg/kg i.p. injection of cocaine. The results of Experiment 1 indicate that the continuous infusion group demonstrated a dose dependent suppression of locomotor behavior by single doses of NANN-190. NANN-190 had no consistent dose dependent effect on the locomotor behavior of the subjects in the other pretreatment groups. The results of Experiment 2 indicate that NANN-190 generally had a greater suppressive effect on cocaine induced locomotion in the daily injection group and terminal 5-HTγ receptor sensitivity. In contrast, NANN-190 had no suppressive effect on cocaine induced locomotion in the continuous infusion group, which is consistent with 5-HTγ receptor supersensitivity. Changes in 5-HTγ receptor sensitivity may mediate the withdrawal symptoms of anxiety and depression exhibited by human cocaine abusers thus providing a potential basis for treatment. This research was supported by NIDA grant SRDC SPSDO-DA55030-02.

516.7

5-HTγ ANTAGONIST INHIBITION OF COCAINE-INDUCED BEHAVIOR AND SEROTONERGIC INNERVATION OF DISTINCT ANATOMICAL SITES. A. Toyoshi and R. Hitzemann, Departments of Psychiatry and Psychology, SUNY at Stony Brook, NY 11794-8101 and VAMC, Northport, NY 11768.

We have previously reported that endogenous 5-HT is required for the development of cocaine-induced hyperactivity (Soc. Neurosci. Abs. 348.16, 1991). It has been suggested that both the caudate-putamen (CP) and nucleus accumbens (NA) are anatomical sites of action for 5-HT, antagonism of dopamine (DA) mediated behaviors (Blandina et al., 1988; Carboni et al., 1989, Chen et al. 1990). In order to further investigate the requirement of endogenous 5-HT in the 5-HTγ antagonist inhibition of cocaine-induced behavior and 2) to localize possible anatomical sites of action, p-chlorophenylalanine (PCPA) treated animals were observed in a longitudinal-recovery study. Animals were pretreated with PCPA (100 mg x 3 days) and then either sacrificed on days 0, 7, 14, 21, and 56 or tested behaviorally for the 5-HTγ antagonist cocaine interaction. Recovery of 5-HT levels in the NA and CP and the raphe nuclei was followed by immunohistochemical techniques. There was no apparent differential recovery of 5-HT in the CP and NA; the rate of recovery was slow with 5-HT levels being only 30 to 50% of normal by day 14. As expected, recovery of 5-HT in the raphe nuclei occurred more rapidly. Data will be presented paralleling 5-HT recovery with 5-HTγ antagonist behavioral efficacy.

516.8


With repeated exposure, rodents become sensitized to the motor-activating properties of cocaine. Cocaine is known to interact with the 5-HT systems in the brain, and cocaine sensitization appears to be associated with an enhanced sensitivity of 5-HT dorsal raphe (DR) neurons to systemic cocaine, fluoxetine and 8-OHDPAT. The present study is designed to compare the sensitivity of somatodendritic autoreceptors to local application of 5-HT in cocaine-sensitized vs. control rats. Male rats were treated with IP saline (n=6) or cocaine (15 mg/kg; n=5) BID for 7 days. Comparisons of behavioral ratings (Kübler-Elwood) between Day 1 and Day 7 indicated that sensitization occurred at 5-HT1 neurons. Following a 24-h withdrawal, rats were anesthetized with urethane (1.5 g/kg) and single-unit extracellular recording and iontophoretic studies of DR 5-HT neurons were conducted. Both ['H]5-HT (1.3 Hz) and 'H]saline (21.2 Hz) in controls exhibited similar current-response curves to DR 5-HT (2.5-10 A; 8.04 M, pH 4.0); an average inhibition of 52.8% was observed at 7.5 A. In contrast, the responsiveness of NA neurons from cocaine-sensitized rats appeared to be dependent upon basal firing rates. Slow cells in cocaine-sensitized rats were more responsive to 5-HT than fast cells from cocaine-sensitized rats as well as both fast and slow cells from saline rats. For example, the initial suppression observed at 7.5 nA of 5-HT was 79.9 ± 5.7% in slow cells and 35.8 ± 6.0% in fast cells. From cocaine-sensitized rats. These preliminary studies suggest that 5-HT DR neurons become sensitized to local application of 5-HT after repeated cocaine exposure. Thus, the enhanced sensitivity observed in response to systemic cocaine, fluoxetine and 8-OHDPAT in cocaine-sensitized rats appear to be associated with at least the level of the cell body. Supported by DA 05708, DA 0611 and NARSAD.
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THURSDAY AM

DRUGS OF ABUSE: COCAINE'S INTERACTION WITH NON-DOPAMINE SYSTEMS

1239

S16.9

SEROTONIN LESIONS AFFECT RESPONSEING ON A PROGRESSIVE RATIO SCHEDULE REINFORCED BY EITHER INTRAVENOUS COCAINE OR FOOD. E.A. Lok*, G. Baker*, G. Vickers* and D.C.S. Roberts†. †Dept. of Psychology, Carleton University, Ottawa, ‡Dept. of Psychiatry, University of Alberta, Edmonton, Canada.

We have shown that injections of the serotoninergic neurotoxin 5,7-DHT into the amygdala or the medial forebrain bundle of rats will result in an apparent increase in motivation to self-administer cocaine (Psychopharmacology 101 (1990) 262). In the present study we examined the effect of intravenous cocaine (0.25 mg/kg; 5,7-DHT on cocaine self-administration reinforced under a progressive ratio (PR) schedule of reinforcement. On this schedule, the response ratios escalate following each reinforcement. Rats were implanted with chronically indwelling catheters and trained to self-administer cocaine on a PR schedule. Rats then received icv infusions of either 5,7-DHT (10 µg; n=10) or acetic/saline vehicle (n=5) and were tested for an additional 7 days. Lesioned rats demonstrated substantial increases in final ratio attained and decreases in forebrain 5HT levels. In a second study, similar increases in final ratio were found in animals responding for food reward following identical 5,7-DHT lesions. The data indicate that 5,7-DHT lesions may produce a global influence on reinforcement rather than a specific effect on cocaine reinforcement. (Supported by the MRC)

S16.11

UP-REGULATION OF SIGMA BINDING SITES FOLLOWING REPEATED EXPOSURE OF GUINEA PIGS TO COCAINE. L. Stein* and Y. Itzhak. Dept. of Biochemistry & Molecular Biology, Repscend Labs, University of Miami School of Medicine, Miami, FL 33101

Although it is believed that blockade of the dopamine (DA) transporter by cocaine is primary for its psychotrophic effects, the drug interacts with several transporters and CNS receptors. Cocaine, for instance, has similar affinities for the DA transporter and sigma binding sites (ca. 1 nM). The later are postulated to be involved in the effects of various psychotrophic agents, and are down-regulated following exposure to the antipsychotic agent, haloperidol (Itzhak & Stein, Brain Res. 506: 166, 1991), that has similar affinities for DA and sigma receptors (ca. 2 nM). Insufficient at both cocaine and haloperidol interact with sigma binding sites, but affect DA neurotransmission in “opposite directions”, we sought to investigate the effects of cocaine-exposure on sigma binding sites. Guinea pigs were treated with either saline or cocaine (40 mg/kg/day; i.p.) for 7 days, sacrificed 24 h following the treatment, and the brain was dissected and prepared for sigma-sigamad receptor binding assays. Binding of (+)-pentazocine and (+)-N-ethyl-N-[(1RS,2RS)-2-(3-hydroxyphenyl)-1-pyrrolidinyl]propanol (PPP), two selective sigma ligands, in various brain regions indicated a significant up-regulation (142% of control) of sigma binding sites in the substantia nigra. In other brain regions no significant change in the binding parameters of the sigma-ligands was detected. The specific alteration in sigma binding sites observed in substantia nigra may be related to the behavioral sensitization that usually follows repeated exposure to cocaine. Supported by NIDA DA07589.

S16.12


We have previously reported that 14 days of either continuous or intermittent exposure to cocaine yields regimen-specific neuroanatomically-selective changes in basal NT-like immunoreactivity (NT-LI) in rat substantia nigra (NT-LI) (Cain et al., Soc. Neurosci. 1991). We have now evaluated residual alterations in NT responsivity in response to a challenge stimulation. Rats were treated with 40 mg/kg cocaine/day for 14 days using continuous or intermittent administration paradigms. After 7 days of withdrawal, the animals were given a 20 mg/kg ip. challenge dose of cocaine and sacrificed 24 hrs. later. In rats treated continuously with cocaine, NT-LI was significantly increased in the nucleus accumbens 24 hrs. following the challenge dose relative to animals treated for 14 days with saline vehicle or intermittent cocaine injection. In contrast, in animals treated with chronic intermittent injections of cocaine, NT-LI in the substantia nigra was significantly increased relative to animals treated for 14 days with saline vehicle or continuous cocaine. These results indicate that differential cocaine dosing protocols induce neuroanatomically-selective changes in the responsivity of CNS NT systems and thus, support the possibility that NT is an important modulator of the behavioral consequences of chronic cocaine administration. Supported by NIDA DA-05303.
EPILEPSY: BASIC MECHANISMS IV

517.2


The potent GABAα blocker picrotoxin, applied to guinea-pig hippocampal slices, induces in the CA1 region an epileptic event called an "afterdischarge" (AD). ADs consist of a long initial (1') burst, lasting up to several hundred ms; a number of brief secondary (2') bursts at 50-65 ms intervals, and a long afterhyperpolarization (AHP). All AD components are synchronized between cell body, apical dendrites, different cells, and the local field potential.

We constructed a model of the disintegrated CA3 region with 100 19-compartment pyramidal cells, each with Na, K and Ca currents whose kinetics were determined (where possible) from whole cell patch records in isolated cells (Traut, Wong, Miles & Michelson, J. Neurophysiol. 1991, 66: 35-50). Cells were randomly connected by excitatory synapses with "AMPA" (fast, voltage-independent) and "NMDA" (slow, voltage-dependent) synapses. The model generates ADs as follows: AMPA synapses mediate the initial synchrony and depolarize the dendrites, unblocking NMDA receptors. The long 1' burst results from the delayed onset of the AHP (Lancaster & Adams, J. Neurophysiol. 1986, 55: 1268-1282). A prolonged NMDA current generates repeating dendritic Ca spikes, producing the 2' bursts. 2' bursts are synchronized in turn by the AMPA synapses. Experimental results supporting this model include (1) effects of NMDA blockers (superseding the 2' bursts), (2) effects of dendritic current injection: a rhythmic series of brief bursts with slow action potentials (presumed Ca spikes).

In conclusion, in this type of experimental epilepsy, recurrent excitatory synapses engage intense cellular oscillatory properties. Recurrent excitation also maintains synchrony of the oscillations.

517.3

EXCITATORY SYNAPTIC COUPLING BETWEEN GABAERGIC INTERNEURONS IN THE HIPPOCAMPUS. H.B. Michelangeli and R.K.S. Wong, Departments of Pharmacology, SUNY Health Science Center, Brooklyn, New York 11203.

We are interested in the modification of synaptic events leading to electrically induced epileptic discharges. Intracellular recordings were obtained from CA3 pyramidal cells in guinea pig hippocampal slices; stimuli (polar, 35-30mV, 140μA) were applied to the stratum radiatum at the border between CA1 and CA2. These stimuli presumably activated the recurrent synapses between the CA3 pyramidal cells. The typical response to a single (test) shock consisted of an EPSP followed by a compound IPSP. Successive tetanization (60Hz; 2s, intensity 2x test stim) progressively altered these synaptic events, ultimately resulting in evoked burst discharges. Specifically, we noted that tetanization induced long-lasting depression of the early IPSP. This could result from either a reduction in the IPSP conductance (g) or an increase in the concomitant EPSP, or both. To determine whether gIPSP is reduced, we examined the test response at different membrane potentials. The IPSP amplitude varied linearly with the membrane potential. Following tetanization of adequate intensity, the slope of this relationship decreased, suggesting a decrease in IPSP conductance (cf. Steirer et al, Nature 1987). This decrease was cumulative with successive tetani. With sufficient suppression of inhibition, delayed EPSPs first emerged most typically between the peaks of the early and late IPSPs. Additional tetani often induced evoked (and occasionally spontaneous) synchronized bursting consisting of an initial component lasting up to 110ms, followed by phasic bursting at approximately 15Hz. These results suggest that the reduction of inhibitory conductance following tetanization allowed recurrent excitatory synapses to sustain epileptiform activity in the CA3 region.

Supported by the NIH and the Consortium for Medical Education in Developmental Disabilities.

We had reported that modest elevation of extracellular K⁺ (8.3 mM) induced seizures (SZs) in organotypic hippocampal(HP) cultures more reliably than in adult HP slices. The literature suggests that HP orgotypic cultures grown in higher temperature show more spontaneous epileptiform discharges. We examined the effect of temperature during the culture on K⁺ induced SZs in organotypic HP cultures.

Organotypic cultures were prepared from 6-day-old rat HP according to the methods of Gähwiler (1988, TINS). Cultures were maintained in a 35°C or a 37°C incubator. At 9-14 DIV, extracellular field recordings were made from CA1 pyramidal layer in standard aCSF (95/4, O₂/CO₂) at 36°C.

Only 6 of 24 cultures grown at 35°C displayed SZs in high K⁺ (8.3 mM) media, while 38 of 43 grown at 37°C exhibited SZs (P < 0.0001, Fisher’s Exact). The SZ frequency and duration were not significantly different between these two groups (35°C mean=5 vs 37°C mean=33; 1.7 ± 6.2 vs 0.3 ± 5.1 days). The SZs were more reliably seen after 30 days postnatally. In CA1 area, interictal discharges had significantly smaller amplitude than in CA3 and SDs had larger amplitude and duration and also, occurred more frequently.

The data indicate that the spontaneous discharges induced by TEA in the CA3 subfield of the rat hippocampal slices display patterns of activity that are dependent upon age. Furthermore, the participation of non-NMDA and NMDA receptors in the TEA-induced activity undergoes age-dependent changes.
517.11
CNQX-SENSITIVE SPONTANEOUS EPSPS AND EPILEPTIC BURSTS IN LOW Mg²⁺ IN RAT HIPPOCAMPAL SLICES
Low concentrations of Mg²⁺ in cerebrospinal fluid have been shown to elicit spontaneous epileptic burst discharges. Here we examined the relationship between spontaneous epsps and epileptic bursts in this model.

Hippocampal slices in vitro were prepared from adult male Sprague-Dawley rats. Intra- and extracellular recordings were made of spontaneous activity in area CA1 in the presence of 1 mM Mg²⁺. APV-sensitive component of glutamatergic excitation was isolated from the fast component by bath application of 20 μM 6-cyano-7-nitroquinolinoxide-2,3-dione (CNQX). In the presence of CNQX no fast epsps could be evoked at either 0 or 1 mM Mg²⁺. Spontaneous epileptic bursts occurred in 0 mM Mg²⁺ (mean ± SEM frequency 7.1 ± 1.0 Hz, n=7) and persisted in the presence of CNQX at a reduced rate (0.2 ± 0.1 Hz, n=5). In the absence of Mg²⁺ two distinct populations of spontaneous epsps were seen with modal half-widths of 6 ms and 14 ms. The short duration epsps were seen alone in the presence of 1 mM Mg²⁺ and were blocked by CNQX. The longer epsps persisted in the presence of CNQX at a reduced frequency. These observations show that APV-sensitive glutamatergic excitation alone was sufficient to generate epileptic bursts. The occurrence of bursting correlated with long-duration spontaneous epsps suggesting that the initial trigger for these epileptiform events was synaptic activation of the NMDA subtype of glutamate receptor.

Supported by the Welcome Trust

517.13
EPILEPTIFORM ACTIVITY INDUCED BY VERATRINDE IN CA1 NEURONS IN RAT HIPPOCAMPAL SLICES. L. M. Tian* and K. A. Alkadhi, Department of Pharmacology, University of Houston, Houston, TX 77204-5515.
Epileptiform activity can be generated in individual neurons without synaptic involvement. The intrinsic mechanism for this is unclear. Using combined intra- and extracellular recording techniques, we report here results showing that abnormality of Na⁺ channels may lead to epileptiform discharges or even seizure-like events in rat hippocampal CA1 pyramidal neurons in vitro. Veratridine-induced Na⁺ channel abnormality is utilized as a model.

In the presence of veratridine (30-100 nM) the action potential duration induced by a depolarizing current pulse is followed by a slow depolarizing plateau (SDP, 6 neurons). Tetrodotoxin (TTX, 10 nM) markedly blocks the SDP amplitude with no significant effects on the amplitude and duration of the initial action potentials (4 neurons). When Ca²⁺ concentration is doubled in the superfuse, the SDP amplitude decreases. The amplitude and duration of SDP decrease when the membrane is hyperpolarized and increase when depolarized.

A burst of action potentials residing on the SDP appears when concentrations of veratridine over 100 nM are used (20 neurons). This burst is paroxysmal and can be blocked by TTX (30 mM) as well as by high Ca²⁺ concentrations, apparently, by decreasing the SDP. These results suggest that an abnormality of Na⁺ channels could be an intrinsic mechanism for epileptogenesis.

517.15
EFFECTS OF ESTRONE-3-SULFATE ON SYNAPTIC CURRENTS IN CA1 PYRAMIDAL CELLS IN THE RAT HIPPOCAMPUS. R. Ramak, D. Crockett, A. McGinty, and S. Voregge*, Dept. of Biological Sciences, San Jose State University, San Jose, CA 95192-0100.
Previous work has demonstrated the convulsant nature of the estradiol metabolite dienestrol in this study, we used whole-cell, thick slice, patch clamp techniques to observe the action of Estrone-3-Sulfate on synaptic currents in hippocampal CA1 neurons of 18-25 day old rats. Synaptic currents were evoked orthodromically by stimulating the Schaffer Collateral pathway. Patch clamp recordings were made using 3-6 MΩ electrodes filled with 125 mM potassium gluconate. Evoked currents were observed at holding potentials ranging from -70 to -55 mV. Stimulation of the Schaffer Collaterals elicited the typical EPSC (inward current)/IPSC (outward current) response. Perfusion of Estrone-3-Sulfate, at a pharmacological concentration (450 μM), produced a decrease in the amplitude of the inward current and a decrease in the outward current. In some cases, however, there was a more sudden transition to a prolonged, multigastric inward current (EPSC) that was not preceded by a decline of the IPSC. In addition, pyramidal cells exposed to ES exhibited both evoked and spontaneous bursting activity. Bursts were associated with a large amplitude inward current that was similar to currents associated with other forms of epilepsy. These data suggest that Estrone-3-Sulfate may either facilitate excitatory synaptic transmission or depress inhibitory transmission. However, further experiments need to be performed to clarify the mechanism by which Estrone-3-Sulfate produces epileptoid activity.

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517.12

Injecting cholera toxin into rat hippocampus induces an epileptic syndrome lasting 7-10 days. Epileptiform activity is preserved in the hippocampal slice in vitro. We have attributed the epileptogenesis to a depression of intrinsic potassium currents responsible for accommodation and for AHPs. Here we measure both spontaneous and evoked postsynaptic potentials (pSPs) to assess synaptic excitation and inhibition.

Cholera toxin (0.5-1.0 μg) was injected bilaterally into dorsal hippocampus of anaesthetised adult male Wistar rats, which were then allowed to recover. Transversal slices of CA1 were prepared 3-4 days later, at the peak of the syndrome, exhibited both evoked and spontaneous epileptiform discharges. When these all-or-none bursts were blocked by raising Ca²⁺ in the bathing medium from 2 to 6 or 8 mM we were able to evoke epsp - ieps sequences. These consisted of a fast epsp followed by both a fast and a slow ieps. The times to peak of the epsp and slow ieps did not differ from controls (p>0.05), although the fast ieps peaked slightly later (p<0.02). Reversal potentials did not differ significantly from controls. No changes were apparent in the conductance underlying the evoked epsp. Initial analysis of spontaneous epsp's indicated a slight increase in epsp amplitude but no change in half-width.

Models of epilepsy where inhibition is intact, such as that induced by cholera toxin, may provide new insights into mechanisms of the epilepsies.

Supported by the Welcome Trust.

517.14
The activity of individual cells in the hippocampus has been modelled using a similar approach to that of Traub (1991, J. Neurophys., 66, 635-650). This model includes a description of the cellular channel currents I_{Na}, I_{KCa}, I_{Kleak}, I_{L} and I_{Lpot}.

However, the kinetics of the processes were empirically tuned to fit bursting behaviour of CA3 and CA1 pyramidal cells.

The pyramidal cells and other populations of interneurons in CA1 have been incorporated into a 3D model of the synaptic connectivity of the CA1 and CA3 areas. Mechanisms of NMDA and NMDA EPSCs, as well as IPSCs mediated by GABA, are included. Bursting in CA1 is driven by CA3, or bursting in CA1 following the synaptic plasticity reported following KA injection (Wheat, 1989, Comp Biochem Physiol., 93A, 211-220) has been investigated.

The simulations of the hippocampal network are performed on transputers using software that is both fast and interactive. A general purpose numerical integration routine and an efficient communication scheme have been developed to allow the model to be run on terascale transputer arrays of different sizes. For example, 100ms of behaviour on a network of 640 neurons can be simulated in 8 minutes using a Parcyc Multichip with 19 Janus T800 transputers.

Preliminary studies indicate that this anatomically based model can be used to study the dynamic properties of neurons in the hippocampus, including monitoring the behaviour of subpopulation of cells that contribute to epileptiform activity.

This project is funded by the M.R.C. and Wellcome Trust.

517.16
Seizure susceptibility in rats peaks during the second postnatal week and declines thereafter toward adult levels. We hypothesized that this may be due to age-related differences in hippocampal sensitivity to elevations in [K⁺]. Hippocampal slices (400-500 μm thick) were prepared from rats of four different age groups; 9-12, 16-22, 28-32, and 60-80 days old (d.o.) and maintained in vitro for 3-24 hours. 

Field potentials were recorded in the CA1 pyramidal cell layer in response to stimulation of the Schaffer Collaterals with constant current pulses (10 msec; 50-250 μA). [K⁺]e was increased stepwise from 3.5 mM to 6 mM, 8.5 mM, and 11 mM, to evoke a depolarizing shift in the amplitude of the extracellular field potential and to increase the number of evoked population spikes increases in rats ≥18 d.o. as [K⁺]e increased, with a peak response at 8.5 mM K⁺. In contrast, spike amplitude decreased in 9-12 d.o. rats, or was blocked completely in association with a large negative extracellular potential. However, evoked responses recovered more readily in slices from younger rats upon washing with 3.5 mM [K⁺].

Spontaneous epileptiform activity occurred in elevated [K⁺]e in 50-80% of slices from rats ≥18 d.o. but not in 9-12 d.o. rats. Our results show that regulation of [K⁺]e is less effective in the hippocampal CA1 region of 9-12 d.o. rats than in adults, probably because of poorly developed metabolic buffering. However, 18-22 d.o. rats appear to be most susceptible to develop epileptiform activity in response to rises in [K⁺].
517.15
THE METABOTROPIC GLUTAMATE RECEPTOR CONTRIBUTES TO THE KINDLING OF EPILEPTIFORM EVENTS IN RAT HIPPOCAMPAL SLICES. S. M. Blevins, W. M. Saltmeyer, A. R. Sheppard and W. R. Adley. Loma Linda University and VA Medical Center, Loma Linda, CA 92357. We previously demonstrated that repeated sine wave stimulation (SW, 60 Hz, 100–600 μA 1–4 pulses, every 5 min) in the CA2+ stratum radiatum kindled afterdischarges (ADs) and electrographic seizures (EGSs) in slices cultured in vitro and CA2+ /CA3. Spontaneous interictal bursts (ISIs) developed 2–4 min following SW induction presenting with ISIs and EGSs requiring activation of NMDA receptor, while the expression of kindled ISIs required activation of non-NMDA ionotropic glutamate receptors. We used the same kindling technique to study the role of the metabotropic receptor (mGlu) in SW–induced epileptiform events. We compared kindling in control medium (ACSF) with kindling during bath applications of the mGlu agonist at-ACPD (10 μM), BMY-2150 (100 μM), and a Ca2+–dependent K+ current blocker (TEA, 50–100 μM).

T–ACPD slightly facilitated ADs and EGSs. However, the initial IS frequency remained unchanged during the first 16 min of spontaneous bursting, was twice as high as in ACSF (0.06–1.12 Hz), stabilized at about 0.1 Hz, and persisted for hours following the last SW. TEA also facilitated SW–induced ADs and EGSs. The initial frequency of ISIs was 20% higher than in ACSF and remained unchanged for 120–180 min. Addition of APs to ACSF or TEA prior to kindling did not alter ADs and EGSs. However, APs reduced the initial frequency of ISIs by about 40% and the bursts disappeared in 60–70 min. By contrast, addition of APs following kindling did not alter the IS frequency. These results suggest that mGlu1 does not significantly contribute to the short term effects of SW (depolarizations, ADs, EGSs), it is not required for the maintenance of established bursts, but it activates long–lasting bursting mechanisms during the induction phase of the ISIs.

517.19
A GABA-WITHDRAWAL SYNDROME IN HIPPOCAMPAL SLICES. Garcia-Ugalde G, Galarraga E, Bargal J and Brailovsky S. Instituto de Fisiología Celular. UNAM. México DF, D.F. The interruption of intracortical infusion of GABA induces electrographic seizures in rats (Brain Res 442:175, 1988). This phenomenon was named “GABA withdrawal syndrome” (GWS). We now describe this phenomenon in vitro. Field potentials in slices of CA1 were taken from the 1st hippocampal slice to study the effects induced by the interruption of GABA superfusion. Also, GABAergic inhibition was examined using the paired pulse paradigm: e.g., when GABA was incubated in GABA (1.5 mM) for 1 or 2 hours. In the washing period, the “intensity vs response amplitude” relationship was analyzed. In some experiments the whole procedure was performed in the recording chamber, that is, the same slice was used before, during and after GABA superfusion (1.5 mM).

With the stimulation parameters used (0.2 Hz, 200 μsec), activation of the Schaffer collateral inputs were used to determine the population spike in control conditions (n=9). In contrast, multiple population spikes were observed in 70% of the slices previously incubated in GABA (5 mM). Also, we recorded an increase (210%) in the population spike with respect to its control value (n=5). This effect was even more evident after 1 hour of GABA washout. A marked reduction of the paired pulse inhibition was recorded in the slices previously incubated in GABA (1 mM) for 2 hours (n=4).

The results indicated that the interruption of GABA superfusion induces hypersynchronous activity in hippocampal slices. This effect may be due to a dysfunction in GABAergic neurotransmission. This work was partially supported by DGAPA-UNAM.

518.1
CORRELATIONS BETWEEN COGNITIVE IMPROVEMENTS AND PHARMACOKINETICS DURING TREATMENT WITH BMY-2150 IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE (DAT). A. Berardi, S. Ashina, K.C. Raffaeli, U. Havry, H. Stratman, K. Pandurangan, T.J. Squires. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892 and Bristol-Myers Squibb Pharm. Res. Inst., Wallingford, CT 06492-7660. We have previously reported that treatment with BMY-2150 in DAT leads to significant improvements on visuomotor and attentional tasks, namely on a simple reaction time task (p = 0.05; Bristol-Myers Squibb report). These results are in line with clinical trials of patients with DAT. Moreover, we have shown that two major active metabolites, BMY-42191 and BMY-40440. Nine DAT patients (mean age = 71 ± 4.03, range 59–96; proband p = 0.05, Bristol-Myers Squibb report) and 10 healthy controls (mean age = 52 ± 5.69, range 45–67; proband p = 0.05, Bristol-Myers Squibb report) were assessed by an extensive psychometric battery at various doses of BMY-2150 (200–900 mg/day); all doses were combined for the statistical analyses. One subject was excluded from the study because of a lack of data. A total of 10 patients were studied at the end of the treatment period. In addition, four patients did not complete the study. The results of the statistical analyses were consistent with the hypothesis that high positive correlations were found between levels of BMY-2150 and both major metabolites (all p = 0.0001). Second order partial correlation analyses were used to determine whether significant cognitive improvements could be accounted for by BMY-2150 or one of its metabolites separately, rather than its total exposure. Significant positive correlations were found between BMY-2150 and all metabolites (BMY-40440) (r = 0.48, p < 0.01). Significant negative partial correlation was found between BMY-2150 and the plasma concentration of BMY-42191 (r = -0.56, p < 0.005). These results suggest that cognitive improvement during treatment with BMY-2150 in DAT patients correlates with kinetic measures and that it may result from the combined effect of BMY-2150 and its two major metabolites.

518.2
TOXICITY DURING CONTINUOUS INTRAVENOUS ADMINISTRATION OF PHYOSITIGME IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE. S. Ashina*, K.C. Raffaeli, A. Berardi, M.D. Schapiro, T.J. Squires. Unit on Pharmacology and Pharmacokinetics, Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD. Alzheimer’s disease (AD) is accompanied by a depletion of presynaptic cholinergic markers that is not well understood in cognitive impairments. In an attempt to improve cognition in AD, physostigmine, a reversible cholinesterase inhibitor, was administered by continuous intravenous infusion to 9 patients with possible or probable Alzheimer’s disease. Escalating doses (0.5–25 mg/kg/day) were administered in a randomized, double-blind, placebo-controlled, cross-over study. First, development of nausea, vomiting, headache, nightmares, sweating, generalized malaise or dizziness during the escalating or double-blind phase. Two of these patients could not tolerate the higher range of doses and the other three developed toxicity when the optimum dose was administered during the double-blind phase. These results suggest that steady-state administration of physostigmine is associated in some subjects with toxicity that may limit its use as a potential therapeutic agent.
518.3

Since the cholinergic system is compromised in Alzheimer's disease, we have compared the responses of 6 patients with mild to moderate dementia of the Alzheimer type (DAT) on treatment with continuous intravenous infusions of physostigmine (a cholinesterase inhibitor) or arecoline (a central cholinergic agonist). All patients were in a separate inpatient study. Patients received infusions of escalating doses of arecoline or physostigmine. Response was tested after 25 and 40 mg/day of arecoline or at 1, 4, 16, 28, and 40 mg/day of physostigmine. Patients' responses to the two drugs were not always consistent. Some subjects improved their performance with at least one dose of arecoline but not with any dose of physostigmine; two subjects improved with at least one dose of physostigmine but not with any dose of arecoline; one subject improved with both drugs; one subject did not improve with either drug. Verbal memory improvement following arecoline tended to occur at low doses (4 or 16 mg/day), while improvement following physostigmine was more likely to occur at high doses (25 mg) which also were likely to cause adverse side effects. Differences in the therapeutic/toxic dose ratio may account for some differences in response to the two drugs.

518.5
SUT-8701, CCK8 ANALOG, HAS POTENTIAL AS AN ANTIDEMENTIA DRUG FOR ALZHEIMER'S DISEASE. K. Sugaya*, M. Takahashi, K. Kojima, T. Kato*, M. Ueda and K. Kubo*. Aging and Intractable Diseases Area, Res. Inst. for Biosciences, Tokyo Medical and Dental University, Faculty of Pharmaceutical Sciences, Science Univ. of Tokyo, 2669 Noda, Chiba 278, JAPAN.

Alzheimer's disease (AD) patients have severe degenerations of cholinergic systems in their cerebral cortices. We reported that cholecystokinin octapeptide (CCK8) prevented the decline of cerebral cholinergic markers in the basal forebrain (BF) lesion rat as a model animal of AD. In this study, we compared the action of CCK8 and SUT-8701 in the several experiments. Continuously s.c. administered SUT-8701 dose dependently preserved the K+ stimulated ACh release and cholinesterase activity. In the pre-training and memory experiments were performed using Morris water maze. We used young and aged rats. Seven days after the pre-training (3 trials a day), half of the young rats were lesioned. After 2 weeks drug treatment, we examined the each group again in the same paradigm. The goal latency of aged or lesioned rats was longer than the young control rats. With the SUT-8701 treatment, the goal latency of aged or lesioned rats was decreased as the young control rat level. The affinity to the CCK receptor of SUT-8701 compared to that of CCK8 was 80 times less in the guinea-pig pumecore, but only half in the mouse cerebral cortex. SUT-8701 had about 100 times less effect on the suppression of liquid food intake, which supposed to be a major peripheral type side effect, than CCK8. These results suggest that SUT-8701 has more potent and selective preventing effect of the degenerations of cholinergic system compared to CCK8 and potential as an antidementia drug for AD.

518.7

In rats, A64A selectively and irreversibly reduces cholinergic markers resulting in cognitive deficits (Walsh et al., Br. Res., p. 91, 1984), which is considered a useful model of AD. Replications of A64A-induced cognitive deficits in rats after acute administration of cholinomimetics (Nakahara et al., Soc. Neuro. Abh., p. 637, 1987) have not been reported. Acute administration of A64A is effective in improving AD memory impairments (Raffaelli et al., Psychopharmac Bull., p. 315, 1991). Chronic administration of cholinomimetics in rats (Nakahara et al.) was of greater benefit. We now report that acute administration of various cholinomimetics to A64A-treated rats did not improve performance deficits on an 8-arm radial maze task. Five daily treatments with ouabain (0.1 mg/kg, i.p.) also did not improve maze performance; however, two-week infusion of arecoline (1 mg/kg/day) or CCK8 (1 mg/kg/d) via osmotic mini-pumps produced significant transient improvements. Two-week infusion of A64A or pilocarpine did not improve performance. Thus, in the A64A model, acute treatment is ineffective. Continuous administration of A64A or cholinomimetics may be inadequate to reduce cognitive impairments. Chronic treatment with cholinomimetics in this model, as in SDAT, appears to be of greater benefit in attenuating cognitive deficits.

518.4
DIPHROPYRIDINE AND PHENYLALKYLAMINE BINDING TO CA++ CHANNELS IN HUMAN NEUROLOGICAL DISORDERS AND AGE-IMPAIRED ANIMALS. A. Sp, T. V. Den*, D. Czyzy, W. Rows, P. Pokuja and R. Qirin. Douglas Hospital Research Centre, and Dept. of Psychiatry, Faculty of Medicine, McGill University, Montreàl, Québec, Canada H2H 1R3.

Alterations in Ca++ availability and metabolism have been suggested as possible mechanisms of cellular aging. Particularly pertinent to the Ca++ availability is the recent demonstration that L-type Ca++ channel blockers, such as nimodipine, can facilitate learning and memory in animals. We have investigated the status of these channels in humans neurodegenerative disorders associated with memory deficits, including Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) diseases. Additionally, comparison was made with data obtained in age-impaired and unimpaired (Morris-maze tested) 24 month old rats. [3H]PN20-110 (dipropyridiphosphonate) and [3H]BD-988 (phenylalkylamine) were used as radioligands to evaluate binding parameters to L-type Ca++ channels using either membrane binding homogenate (human) or quantitative receptor autoradiography (rat). Rather surprisingly, and despite neuronal cell losses, it appears that both [3H]PN20-110 and [3H]BD-988 binding are well preserved in HD, PD and HD brain tissues, except for a significant decrement in rmax values in the basal ganglia of HD patients. Specific [3H]PN20-110 binding parameters are also rather similar in various regions of the age-impaired and unimpaired rat brain. Taken together, these results suggest that the L-type Ca++ channel binding protein is well preserved in various neurodegenerative disorders. This also indicates that nicotinic radioligands should be hampered by losses of the relevant Ca++ channel receptor protein. Sponsored by Miles/Bayer.

518.6

Increased hypothalamic-adrenocortical (HAP) axis responsivity has been documented in aged rodents and glucocorticoids have been implicated in age-associated neuronal degeneration. We asked if enhanced HAP axis responsivity occurs in normal human aging and in Alzheimer's disease (AD). The plasma ACTH, beta-endorphin (BE), and cortisol responses to the cholinesterase inhibitor physostigmine were determined in younger normals (n=10, age 71 ± 2 yrs), AD patients (n=11, age 72 ± 2 yrs), and young normals (n=7, age 27 ± 2 yrs). The area under the curve, or index norm, and AD patients had significantly higher ACTH, BE, and cortisol responses (p<0.01) than did young normals. Plasma physostigmine concentrations were similar among groups. Under placebo, beta-endorphin to ACTH was significantly lower in the AD subjects (39 ± 03) than in the older normal (1.24 ± 073) or young normal (1.09 ± 36) subjects. These results suggest that the age-associated enhanced HAP axis responsivity observed in rodents also occurs in humans and that processing, secretion, or metabolism of POMC products may be altered in AD. Supported by AG52136, AG08419, and the Dept. of Veterans Affairs.

518.8

Ryan and colleagues (1990, 1991) have developed an animal model of wandering using bilateral injections of 15 mg/ul colchicine into the rat dentate gyrus. The present set of experiments elaborated on the model by investigating the influence of amphetamine, a dopaminergic antagonist, on activity and lesion duration of lesion effects. Experiment I examined cholinergic and dopaminergic interactions through the use of several agonists and antagonists: MeCamfylamine, Pinocione, Spiperone, SCH 23390, SKF 38393, and Quiniprole. The results indicate that the D1 and D2 receptor antagonists administered simultaneously ameliorated spatial learning and memory deficits. The beneficial effects are significantly enhanced by the additional administration of the nicotinic antagonist. The nicotinic antagonists administered alone worsened circumspection swimming. Dopaminergic receptor agonists have no effect on observed behavior. Experiment II investigated the differential effect of administering 7, 15 and 25 mg/ul colchicine on activity and spatial learning. The results indicate that the highest dose of colchicine consistently induces circumference swimming and this appears to be associated with an increase in activity in the Waterman wheel. Experiment III examined behavior 60 and 120 days postlesion. The results indicated that behavioral deficits are stable by 60 days. By 120 days nonlesioned animals exhibit age related behavioral deficits. The studies confirm the viability of the animal model and have implications for treatment.

Amridin (5,6,7,9-tetrahydro-8H-carbazole-4-carboxylic acid monohydrate hydrochloride) (NIK-247) has been patented as a learning-stimulation and memory-improving drug (U.S. Pat. No. 4,552,923; Lammeneg et al., 1988). It was developed in the former USSR, where it is prescribed for symptomatic treatment of senile dementia of Alzheimer type. Its mechanism of action may be related to activation of cholinergic neuronal pathways and improvement of acetylcholine concentration in the synaptic cleft.

The objective of this study was to investigate the effects of amridin on AChR single channel kinetics. Nicotinic AChR-gated ion channels in clonal BC-4NI1 mouse tumor cells were studied using the whole-cell patch clamp technique. 200 nM acetylcholine (ACh) and 1 to 40 μM amridin were applied at transmembrane potentials of -70 to -140 mV. Amridin reversibly blocked open AChR-gated ion channels in a concentration-dependent manner. In the presence of drug, brief closed periods, or gaps, appeared in ACh-activated single channel currents, transforming them into a burst configuration (Nieher and Steinbach, 1976). The mean channel open time decreased with increasing drug concentration. The amplitude of single-channel currents did not appear to be altered by the drug. Channel blocking may antagonize the cholinergic activating effects of ACh and lead to K+ channel-mediated restoration of a more normal resting membrane potential in pathologically depolarized neurons. The physiological significance of this antagonism would depend on the relative efficacies of the drug at its various sites of action.

518.12 EFFECTS OF LIPIDOPRIDE ON K+ CURRENTS OF SKNSH FIBROBLASTS. D. J. S. Daas and R. J. Potter. Department of Neurology, Harvard Medical School, Boston, MA 02115.

The acute phase protein α-antichymotrypsin (ACT) is a primary component of the chromosomal pattern of Alzheimer’s disease (AD), where it is bound to β-A4 protein, a proteolytic fragment of the amyloid precursor protein (APP), a major constituent of the neurofibrillary tangles, and in situ immunohistochemistry indicates that astrocytes are a source of the increased amounts of ACT in the AD brain. We have previously shown that demethasone, and perhaps some other unknown cytokines, can induce the expression of ACT mRNA in cultured rat astrocytes. Since the acute phase mediators IL-1 and demethasone have been shown to induce ACT mRNA in the HepG2 culture system, we asked whether these factors could induce ACT expression in human astrocyte cultures derived from AD brains. We found that demethasone and IL-1 could cooperatively induce ACT message 20-fold in subconfluent astrocyte cultures derived from the frontal cortex of AD brain. However, astrocyte cultures from the brain stem and cerebellum had a negligible baseline expression of ACT message (even in the confluent stage), and the ACT message levels in these cultures were much lower compared to the induction seen in the cortical subconfluent cultures. These results indicate that the differential response of astrocytes to IL-1 and demethasone may be due to the differential expression of ACT mRNA in different brain regions may underlie the localization of pathology seen in AD: mature plaques are seen in cortical regions of the AD brain, and the deposition of APP + diffuse plaques — followed by progression to mature plaques and neuronal cell death in those regions of the brain where the local glial environment is more likely to develop an inflammatory response.
518.15


Acetylcholinesterase (ACHE) inhibitors are potential palliative therapies for the treatment of Alzheimer's disease (AD). The present report describes the pharmacology of PD 142676, an inhibitor, PD 142676. The new compound is equipotent with tacrine in inhibiting human ACHE (IC50 of 40 nM) and similar in potency to other ACHE inhibitors being considered for the treatment of AD. This ADAC-selected ACHE inhibitor, PD 142676, is a new compound, mixed inhibitor of ACHE (Ki=45 nM, Kii=81nM) that binds in the active site of the enzyme, as determined by protection from irreversible inhibition. PD 142676 binds to all subclasses of muscarinic receptors with nanomolar affinity and may act as an antagonist based on its ability to reverse ameliorated-induced decreases in the release of 3H-ACh from rat cortical slices. The compound also blocks high affinity choline uptake by rat hippocampal synaptosomes with an IC50 of 2.5 μM compared to 9.59 μM for tacrine. These results show that PD 142676 possesses in vitro pharmacological properties similar to its novel chemical structure. The accompanying abstract shows that PD 142676 also has in vivo central cholinomimetic activity.

518.17


Tacrine is currently under consideration for the palliative treatment of Alzheimer's disease. A method has been established to search for proteins with which tacrine may interact in an effort to understand fully its mechanism of action. A tacrine-sepharose affinity gel was synthesized in a single step by coupling it to epoxy activated sepharose through its primary amine. This affinity gel was tested for its ability to isolate acetylcholinesterase (ACHE) from both bovine serum and Torpedo electric organ. ACHE is purified to near homogeneity in a single step from these sources. In addition, several other proteins are purified from these sources and are currently under investigation. In summary, other methods used in the purification of ACHE are often complicated by lengthy isolation protocols and/or by multiple organic synthetic steps needed to produce the affinity gels. The tacrine affinity gel provides a simple method for creating an affinity column for ACHE purification and holds the potential to identify other proteins with which tacrine interacts.

518.19


The combined endocrine and neurotransmitter deficiency hypothesis for the etiology of AD, serve as the basis of our study. The central hypothesis is that ovarian steroids, specifically estradiol (E2), serve a neuroprotective role in the function of the basal forebrain cholinergic system. We studied the effects of ovarian steroid deprivation (by ovariectomy) and estrogen replacement on learning, using the 2-way active avoidance paradigm, at two separate time points. The short term ovariectomized (OVX) and steroid-replaced (E2) groups were ovariectomized for 3 weeks. The E2 group received estradiol replacement for 2 weeks prior to behavioral testing. These same animals were tested for learning 2 weeks later, with estrogen replacement maintained both during the testing period and between short and long term testing points. Our data show that the short term OVX group were learning-impaired and possibly due to their ability to learn 2 weeks later. At the long term testing stage, a significant effect of ovariectomy was observed on total avoidance (or correct responses) made over the testing period when compared to intact controls (24 ± 7.8 vs. 83 ± 24). Estrogen replacement prevented this deficit. Furthermore, the short term E2 group learned the task by reaching a desired criterion in 9.5 ± 2.1 days. Maintenance on estradiol replacement and subsequent estradiol 21 weeks later showed that the animals reached criteria in a much shorter time (1.3 ± 0.3 days), suggesting a significant retention of the previously learned task. In contrast, OVX animals never attained the desired criteria. The method employed in relating estrogen produced plasma levels that were in the physiological range (26 ± 43 pmol, with one exception (estradiol: 26 pmol) is an estradiol level, levels of estradiol play an important role in cognition and memory. This research may provide a novel animal model for the study of AD and supports a role for estrogens in memory and cognition (Supported by NIH AG 10485).

518.20


Intracranial infusion of various toxicants has been used to experimentally produce an animal model of neurodegeneration. The goal of the present experiment was to make direct comparisons of ibotenic acid and colchicine in terms of damage to basal forebrain cholinergic neurons. Bilateral infusion of either colchicine (3.0μg/0.5μL/site), ibotenic acid (6.0μg/0.5μL/site) or vehicle (0μl/0.5μL/site) were made in the nucleus basalis magnocellularis (NBM) of male Long Evans rats (n=150). Four weeks post-injection, behavioral assessments were made. The toxicants produced equivalent decreases in step-through latencies in passive avoidance behavior without causing an increase in locomotor activity. Five weeks post-injection, rats were sacrificed for neurochemistry or histochemistry. ChAT and ACHE activity were measured in several brain regions of half of the rats. Within the frontal and piriform cortices, a significant decrease in activity (25%) was seen in the lesioned rats. The brains of the remaining rats in each group were sectioned and stained using ChAT immunohistochemistry and ACHE histochemistry. Analysis of the number of ChAT-immunoreactive cells in the NBM showed a significant decrease (70%) following infusion of either toxicant. Qualitative ACHE histochemical staining in the NBM and cortex showed a similar decrease following infusion of either toxicant. In summary, similar levels of cholinergic hypofunction were achieved for both toxicants and further comparisons of "extracholinergic" markers are planned.
519.1


Potential bioactivated toxins, 2-N-methyl-8-carboxylic acids and 2, 9-N,N-dimethyl-8-carboxylates, were analyzed in the parietal association cortex of human brains from GCMS. The brains (n=9) were taken from fresh corpses with no history of abnormal neuropathology, during forensic autopsies. 2-Methyl-norharmane existed in all samples (0.16 ± 0.05 pmol/g, mean ± SD) and 2,9-dimethyl-norharmane was detected in 8 of out 9 samples (0.10 ± 0.04 pmol/g). 2-Methyl-norharmane and 2,9-dimethyl-norharmane were detectable in only two samples (0.03 ± 0.07 and 0.05 ± 0.10 pmol/g, respectively). Norharmane and harmane were also measured using HPLC/fluorescence detection. Norharmane was present in all samples (0.56 ± 0.45 pmol/g), whereas harmane was detected in 7 of out 9 samples (0.21 ± 0.07 pmol/g). Generally, the levels of N-methylated 8-carboxylic acids in the brain were lower than those of their non-methylated forms. Recent studies show that N-methylated 8-carboxylic acids resemble the synthetic parkinsonian toxicant, MPP+, with respect to structure and neurotoxic activity (Brain Res. 570, 154, 1992). Such "bioactivated" carboxylum ions could be endogenous causative factors in Parkinson’s disease. Supported by the Japanese Ministry of Education, Science and Culture.

519.2


Phenylethanolamine-N-methyltransferase (PNMT) neurons were mapped in the medulla oblongata from 7 patients with Idiopathic Parkinson’s disease (PD) and 8 age-matched controls. Brains were removed and fixed by perfusing alcoholdehyde by carotid and vertebral arteries. Serial transverse sections (50um) through the brainstem were divided into 15 series. Neuropathological examination of the substantia nigra and locus coeruleus were performed to confirm PD, using conventional criteria. One series of sections was incubated with 1:5000 anti-PNMT, diluted 1 in 10000, and processed using the avidin-biotin-peroxidase procedure. PNMT cells in each section were counted using the Magellan program and a Macintosh 120c computer. The total number of cells was estimated by multiplying the number per section by 15. In the ventrolateral medulla, from the level of the obex to 11 mm ventral to the obex, there were 763±844 C1 neurons in normals. This number was significantly reduced in PD (630±815, P<0.01), 53% loss) and many PNMT neurons contained Lewy bodies. We observed a midline C3 group of PNMT neurons in normal brains and this group was also severely affected (68% loss) in PD. Neither the C2 group nor the small PNMT neurons in the nucleus tractus solitarius was significantly reduced. Our results demonstrate a selective loss of C1 and C3 PNMT cells in PD, providing the first quantitative evidence for the involvement of sympathetic premotor neurons. These changes may underlie some of the autonomic symptoms of PD.

519.3

EXPRESSION OF CORTICAL NICOTINIC CHOLINORECEPTORS IN PARKINSON'S DEMENTIA COMPARED TO ALZHEIMER'S DISEASE. H. Schroeder*, E. F. Giacobini, R.G. Struble, A. MacLeish. Dept. Pharmacology and Psychiatry, Southern Illinois Univ. Sch. of Med., Springfield, Ill. 62794, #Dept. Physiological Chemistry and Pathobiology, Univ. of Mann, F.R.G. In Alzheimer’s disease (AD) as well as in Parkinson’s dementia (PD) the neuronal expression of nicotinic cholinoreceptors (nACHr) is significantly decreased (Schroeder et al., Neurobiol. Aging 12:259, 1991) whereas no data are available on nACHr expression in PD cortices. Using the monoclonal nACHr-antibody WB 6 (Fels et al., J. Biol. Chem. 261: 15726, 1986) autopsy samples from human frontal cortex were studied immunohistochemically in: (1) PD patients [n=6; 78±4yrs] and (2) age-matched controls [n=4; 75±5yrs]. Densities of WB6-immunoreactive [l] 267±67 neurons/mm² (mean±SEM) (2) 560±1357] and of crossvlyso-stained neurons did not show statistically significant differences (p>0.05). Although ranges of nicotinic binding sites are similar in homogenate binding studies in similar in AD and PD (Whitehouse et al., Arch. Neurol. 45:722, 1988), in contrast to AD, neuronal nACHr expression appears to be only slightly reduced in PD. This speaks in favor of a qualitative alteration of nACHr binding sites in PD rather than for a shortage of nACHr protein supply. Supported by the Deutsche Forschungsgemeinschaft (Schr 285/11-1), Southern Ill. Univ. Central Res. Committee award and R.J. Reynolds Tobacco Co.

519.4

STRIATAL DOPAMINE, TYROSINE HYDROXYLASE, AND DOPAMINE TRANSPORTER ARE MARKEDLY REDUCED IN A PATIENT WITH DOPA-RESPONSIVE DYSTONIA. X.H. Zhong*, A.H. Raiput, O. Hornykiewicz, and S.J. Kish. Clarke Institute of Psychiatry, Toronto, and Univ. of Saskatchewan, Saskatoon, Canada.

Dopa-responsive dystonia (DRD) is a variant of childhood-onset idiopathic dystonia which shows a therapeutic response to L-dopa. To date, little is known regarding the biochemical changes underlying the disease. We have measured the striatal dopamine (DA) level, tyrosine hydroxylase (TH) activity and protein level, and DA uptake sites (by specific GBR 12935-binding) in the caudate and putamen of one DRD patient (aged 19, first described by Raiput et al., 2nd Intl. Cong. Movement Disorders, Munich, June 24-26, 1992) and four controls matched with respect to age and postmortem time. A marked DA loss (-97%) accompanied by reduction of TH activity (-88%) and TH protein concentration (-79%), and reduction of DA uptake sites (-68%) in putamen was found in our patient, with somewhat less severe changes in caudate (-83%, -45%, -42% and -9% respectively). The extent of reduction was in the pre-parkinsonian range, being less severe than that in Parkinson’s disease (PD). However, the subregional pattern of the reduction was similar to that in idiopathic PD. Our findings are consistent with the excellent clinical response of DRD patients to L-dopa therapy and suggest a cause-effect relationship between the striatal DA loss and the DRD syndrome.

519.5

SUPEROXIDE DISMUTASE EXPRESSION IN THE NEGRO-STRIATAL PATHWAY OF PATIENTS WITH PARKINSON’S DISEASE. A. Bacciu*, C. Thiffault, N. Haman, D. Dias and J. Poirier. Douglas Hospital Research Centre, Department of Psychiatry and Centre for Studies in Aging, McGill University, Montreal, Quebec, Canada.

Several recent in vivo and human studies have provided evidences suggesting a role for the excessive formation of destructive hydroxide peroxide and/or the lack of antioxidant protection in the progression of dopaminergic neurons of the substantia nigra of parkinsonian patients. The most interesting and consistent finding related to the antioxidant status in idiopathic parkinson's disease (IPD) has come from recent different laboratories showing a significant increase in the activity of the superoxide dismutase (SOD) in the striatum and substantia nigra (SN) of patients with IPD. This activity has been widely associated with superoxide detoxification, the end product of the reaction, namely the hydrogen peroxide, has received little attention. Accordingly, we have begun a systematic analysis of the two major forms of superoxide dismutases (copper/zinc and manganese) in terms of activity, protein levels and mRNA prevalence in the substantia nigra and striatum of control and IPD individuals. Results obtained so far indicate that the major portion of the copper/zinc SOD mRNA is restricted to neurons containing neurons of the substantia nigra. In the striatum, the distribution of the copper/zinc SOD is more homogenous and appears to be mostly restricted to neuronal populations. A significant increase in the activity of the manganese form of SOD (but not of the copper/zinc form) was found in the striatum of parkinsonian patients. Supported by the Parkinson Foundation of Canada and by a Scholarship from The Medical Research Council of Canada.

519.6

THE EFFECT OF L-DOPA/2000 AND MPTP ON SUPEROXIDE DISMUTASE ACTIVITY IN THE STRIATUM OF C57BL/6 MICE. C. Siffat*, R. Quinn, M. Ament, and L. Bijvoet. Douglas Hospital Research Centre, Department of Pharmacology and Therapeutics, Department of Psychiatry and Centre for Studies in Aging, McGill University, Montreal, Quebec, Canada, H3G 1R3.

L-dopamine, a potent monoamine oxidase B inhibitor is currently used in the treatment of Parkinson’s disease (PD). L-dopamine appears to delay the necessity of L-dopa therapy in the early stages of the disease although it does not halt the progression of PD. The beneficial effect of deprenyl in PD seems to be of short duration (6-12 months). Recently, it has been demonstrated that administration of L-dopamine results in an increase in the expression of superoxide dismutase (SOD) activity in the striatum. SOD is a key enzyme involved in the detoxification of superoxide radicals and is principally expressed in neurons. Results obtained in our laboratory indicate that acute administration of 100 mg/kg, every 2 days of deprenyl, or deprenyl methyl-4-arylphen-1,2,3,6-tetrahydrodipyrindine (MPTP; 3 X 20mg/kg, every 2 days) over 15 days periods induce an increase in the activity of SOD in the striatum of C57BL/6 mice by more than 2-fold. It appears that the induction of SOD is beneficial to dopaminergic neurons. The MPTP regimen which destroyed 40-60% of tyrosine hydroxylase immunopositive neurons causes a marked decrease in the striatal SOD activity in C57BL/6 mice. These latest results contrast with the increase of the SOD activity as observed in the striatum of PD patients.*

*Supported by Parkinson Foundation of Canada and by Fond de Recherche en Santé du Quebec.
519.7

LOSS OF BASIC FIBROBLAST GROWTH FACTOR IN SUBSTANTIA NIGRA NEURONS IN PARKINSON DISEASE

Basic fibroblast growth factor (bFGF) has been shown to occur in mammalian dopaminergic neurons and to have a neurotrophic effect on these neurons in vitro and in vivo. We examined the substantia nigra (SN) of 6 cases of Parkinson's disease (PD) and 8 controls immunohistochemically using a monoclonal antibody to bFGF. The mean number of melanin-positive neurons in sections of PD SN was 30.2% of the control mean, but the number of bFGF-immunopositive neurons was only 4.7% of the control mean. BFGF-immunoreactivity was observed in only 8.2% of PD, but in 93.7% of control melanin-positive neurons. These results suggest a profound depletion of bFGF in surviving dopaminergic neurons of the SN in PD. This depletion may be related to the disease process. (Supported by the Japan Found. for Aging & Health, the MRC, and the the Parkinson Found. of Canada)

519.9


Levodopa crosses the blood-brain barrier via the large neutral amino acid (LNAA) transporter. LNAA's have been shown to reduce the clinical efficacy of levodopa in fluctuating parkinsonian patients, possibly by competitive inhibition of levodopa transport into brain. To determine whether CSF levodopa is an accurate index of brain levels, we administered phenylalanine challenges during two-hour, constant-rate levodopa infusions and monitored plasma and CSF drug levels in two parkinsonian patients with Omaya reservoir implants in their lateral ventricles. CSF levodopa levels were not reduced by phenylalanine when compared to levels achieved during infusions without the acid challenge. In contrast, the duration of the clinical response to levodopa was markedly reduced from over 100 minutes in the absence of a challenge to less than 20 minutes in the presence of phenylalanine. Phenylalanine had no effect on CSF in one patient, while DOPAC and HVA levels in CSF were not appreciably altered. The plasma and CSF levodopa kinetics with and without phenylalanine were similar to those observed in monkeys, with levodopa entry into CSF lagging behind that of plasma. These results suggest that CSF levodopa is not in equilibrium with brain extracellular or neuronal concentrations and that CSF levodopa levels are not an accurate indicator of clinical response. Levodopa is probably transported into CSF at the choroid plexus, and this transport must differ from that at the capillary endothelium. Supported by NIH Grant NS21062.

519.10


A major route for disposition of levodopa (L-DOPA) in the presence of carbidopa is O-methylation by catechol-O-methyltransferase (COMT). Inhibition of COMT could augment the clinical effects of L-DOPA by decreasing elimination and increasing bioavailability of L-DOPA, and by reducing accumulation of 3-O-methyldopa (3M0D). The acute effects of Entacapone (OR-611), a competitive inhibitor of systemic, and to a lesser extent, brain COMT, on the pharmacokinetics and pharmacodynamics of levodopa has been examined in 7 parkinsonian patients with fluctuating motor responses to levodopa. OR-611 did not affect maximum concentrations of plasma L-DOPA achieved after oral or intravenous administration, nor did it affect the time to maximum concentrations after oral administration. The area under the time-concentration curve was increased by 58% (p<0.04, n=6) after oral administration and by 45% (p<0.01, n=7) after intravenous administration. The elimination rates were reduced by 44% and 34% respectively. The duration of clinical response to L-DOPA was increased by 50% (p<0.06, n=6) with oral doses and 47% (p<0.02, n=7) with intravenous infusions. This results indicate that OR-611 may help in the treatment of parkinsonism by prolonging the clinical response to L-DOPA. This effect is achieved without increasing maximum plasma drug concentrations, which may reduce adverse effects. Supported by NIH Grants NS21062 and RR00334, and Orion Pharmacuetica.

519.12


Complex I (NADH dehydrogenase) is the proximal enzyme of the mitochondrial electron transport chain and is inhibited with high affinity by rotenone. Defective complex I activity has been implicated in the pathogenesis of Parkinson's disease, but little is known of the characteristics or distribution of this enzyme in brain. We have custom-synthesized and radiolabeled a rotenone analog, [3H]dihydrotorenene (DHR), and have used it to develop a quantitative autoradiographic assay for complex I in brain. Using 100 μM rotenone to define nonspecific binding, DHR binding to rat brain is saturable with a K0 of about 10 nM; the Bmax varies more than 20-fold across brain regions. At DHR concentrations below 10 nM, virtually all binding is specific. Scatchard analysis suggests that DHR binds to a single class of sites. The specificity of DHR binding for complex I is indicated by (i) displacement of binding by rotenone and MPP+, (ii) loss of binding after lipid extraction, and (iii) marked enhancement of binding by NADH. We conclude that DHR binding provides a quantitative method to assess the biochemical characteristics of the rotenone binding site of complex I with a high degree of anatomic resolution. (Supported by the Hope Geoghegan Fund, The American Academy of Neurology, The United Parkinson Foundation, and USPHS Grant NS01487).

Parkinson's disease (PD) is characterized by massive degeneration of the dopaminergic neurons in the substantia nigra pars compacta (SN). Moreover, the functional capacity of the surviving nigral neurons is affected, as indicated by a reduction in tyrosine hydroxylase (TH) mRNA in these neurons. Thus, to test the ability of the remaining neurons to express TH protein, a semi-quantitative immunocytochemical method was developed and used to determine the relative amount of TH per neuron on mesencephalic sections of 9 control subjects, 6 patients with PD and 3 with Alzheimer's disease (AD). A second set of experiments was performed on 5 other controls and 5 PD patients for which the neuronal content of TH mRNA has been analyzed previously. Proportionality between the denervation loss and the denervation loss obtained in the operative conditions and the titratable concentrations of TH was established using bovine adrenergic medulla homogenates as standards. Variable amounts of TH were detected in the denervated parts of the disease, and became, in the SN of PD patients, a correlation was observed between the reduction of the cellular content of TH protein and that of TH mRNA, suggesting that the efficiency of TH gene expression is altered in the remaining dopaminergic neurons.

DEGENERATIVE DISEASE: OTHER

520.1 GABAERGIC LOCAL CIRCUIT NEURONS DEGENERATE IN THE MOTOR CORTEX OF AMYTROPHIC LATERAL SCLEROSIS PATIENTS. Kuninobu Niihara, Ann C. McKeel and Neil W. Kowall. Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Recent evidence suggests that neuronal degeneration in amyotrophic lateral sclerosis (ALS) may be mediated by excessive glutamate receptor activation. In the cerebral cortex, GABAergic local circuit neurons resist NMDA-type glutamate receptor mediated excitotoxicity but are disproportionately sensitive to non-NMDA receptor agonists. In Huntington's disease, a distinctive subset of GABAergic local circuit neurons containing parvalbumin are relatively spared, consistent with NMDA receptor-mediated toxicity. We evaluated GABAergic parvalbuminergic neurons in frontal and occipital cortices in the rolandic cortex of six patients with sporadic ALS and six age-matched controls to determine if the pattern of neuronal loss in ALS is consistent with glutamate receptor mediated neuronal injury. In ALS motor cortex, the density of parvalbumin immunoreactive neurons (per mm²) was significantly decreased compared to control values (31.4±3 vs. 52.2±4, p=0.003, unpaired t-test). The depletion of parvalbumin immunoreactive neurons in ALS motor cortex contrasts with the pattern of sparing found in Huntington's disease and is consistent with non-NMDA glutamate receptor mediated neurotoxicity.

520.3 ASTROGLIOSIS IN GUAM AMYOTROPHIC LATERAL SCLEROSIS (ALS). D. Nagay, J. Bransfield, T. Kato, J. Destefano, R. Kurose* ALS Research Foundation, 16301 Pacific Medical Center, San Francisco, CA 94115

Guam amyotrophic lateral sclerosis (GALS) has three salient features which distinguish it from ALS, (1) high incidence rate, (2) dementia, and (3) particular histopathological features common to Alzheimer's disease and Parkinsonism-dementia complex (PDC). Nineteen cases (mid-fatal and temporal cortices), eight GALS, two GALS presenting with PDC (GALS/PDC), five Guam PDC (GPDC) and four neurologically normal Guam cases comprised of persons of Chamorro descent (because of the incidence of neurofibrillary tangles within the population even amongst non-ALS, non-PDC cases), were examined at crossings with and immunoperoxidase technique for glial fibrillary acidic protein (GFAP).

Astrogliosis in GALS reveals itself in similar fashion to ALS in the subcortical white matter. Results indicate the morphological parameters of an ongoing, "reactive" astrogliotic process within the subcortical subcortical white matter in GALS in which gliotized astrocytes have two distinct morphologies; these profiles are located in different areas. The astrocytes of the gray/white matter junction display very numerous, elongated process-plasmalemma contacts; in contrast, regressive astrocytes are evident just deep to this junction. Gray matter astrocytes have smaller cell bodies, shorter processes, and staining is not prominent. Patches of GALS in the presence of gliomas, lead-like structures are found in many linear processes of astrocytes, descending from the glial limits. GALS/PDC cases show more consistency in gliotic response and similarity to GALS cases; conversely, GPDC cases display either numerous intensely stained astrocytes (4 cases) or no gliosis (3 cases). GALS has clinical and pathological differences from ALS as well as individual cases of both astrogliosis implicates a specific role of involvement in the pathology of all forms of ALS.

520.4 GEF AND BETA AMYLOID STAINING IN THE CORTICAL GRAY MATTER IN AMYTROPHIC LATERAL SCLEROSIS (ALS). D. Nagay, T. Kato, J. Destefano, R. Kurose* ALS Research Foundation, California Pacific Medical Center, San Francisco, CA 94115

We examined cryosections of the midfrontal, interior parietal, cingulate, temporal, and occipital cortices, as well as the primary motor cortex from 15 cases of adult-onset amyotrophic lateral sclerosis (ALS) with immunocytochemistry using anti-gliial fibrillary acidic protein (GFAP) and anti-beta amyloid protein. By GFAP staining there was found an increased subpial glial, "patchy" astrocytosis in LI-II, occasionally a few patches in the LIV, and a "finger-like" astrocytosis in LVI-VI. The pathcy astrocytosis was characterized by multiple clusters of astrocytes, each containing 2-8 glial cells, and it occurred in 9 out of 15 cases. The "finger-formations" of the gray/white matter interface seemed to be an extension of the previously described widespread subcortical astrocytosis (Neurophathol J 263:277, 1991) and was found in 11 out of 15 cases. By beta amyloid immunostaining we observed primitive plaques in 5 out of 15 cases. There were no classic plaques. Total number of ALS cases with both astrocyte-patches and amyloid plaques was 14 out of 15. This study demonstrates a ubiquitous patchy astrocytosis in widespread cortical brain regions, suggesting a potential involvement of the whole brain in the pathomechanisms of the ALS. We cannot account for these patches as classic or primitive plaques, although they may indicate possible future beta amyloid production. (Supported by M.D.A.)

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580.5
PROCESSING OF Tau PROTEIN IN Pick's DISEASE. S. Murayama*, Y. Higashi, and H. Mori, Department of Neuropathology, University of Tokyo, Tokyo, 113 and * Kawasaki Medical School, Kurashiki, 701-03, Japan

In Alzheimer’s neurofibrillary tangles (ADNT), tau protein is cleaved in the N-terminal and only its carboxyl third is left (Kondo et al, Neuro 1988:1-827).

We adopted an immunocytochemical approach whereas similar procedure is observed in Pick’s disease (PD) that share immunochemical properties with ADNT. Formalin-fixed, paraffin-embedded hippocampus that were abundantly with PD from four cases of Pick’s disease (PD) were employed for this study. Ultrastructure analysis of these cases contained PD with classic immunocytochemical procedure with occasional twist-profiles. PB-type filaments were also observed in balloononed neurons (BN) and neurone around PB. Immuno-immunocytochemically, phosphorylated epitope of the carboxyl third of tau protein was localized on these filaments (Murayama et al, Ann Neurol 1991:27-394). Employed antibodies and recognized sequence of tau protein were as follows: Alz 50 (2-16); anti-human tau (45-65); tau 1 (392-234); P2 (296-365); tau 3 (534-369); and tau 6 (420-429). Antibodies against both N- and C-term of tau protein visualized BN and neuritic change around PB. However, the staining pattern with anti-N or anti-C-term was different in: 1) anti-C-term stained PB more intensely than anti-N-term; 2) C-term-positive neurites were thicker and more curved than N-term-positive neurites; and 3) C-term-negative area in BN was smaller in size and more crisp than N-term-negative area. The most intense staining was obtained by P2 that recognized a sequence that is tightly bound to the core of paired helical filaments (PHF). These data suggest: 1) first abnormal deposition of tau protein and then change in N-term occur in PD as well as in Alzheimer’s disease; and 2) the core of PB-type filament and PHF may share the same sequence of tau protein.

580.6
PHF POSITIVE NEURITES IN A SEVERE CASE OF PICK’S DISEASE. E.J. Crocker, P.R. Zimmerman*, E. J. Mufson, Department of Neurological Sciences and Pathology, Rush Alzheimer’s Disease Center, Rush-Presbyterian St. Luke’s Medical Center, Chicago, IL 60612.

Pick’s disease (PD) may present with a clinical picture which is indistinguishable from Alzheimer’s disease (AD), although the neuropathological characteristics are significantly different. We report the case of PD with classic immunocytochemical properties with extensive paired helical filament (PHF) containing neurites, without the AD-like senile plaques or neurofibrillary tangles. Gross evaluation of the brain of this 69 year old woman with a 11 year history of progressive dementia showed severe atrophy involving the frontal, temporal, parietal, perisylvian and occipital lobes. Formalin fixed (4%) immersion fixed tissue was either paraffin embedded or cut on a freezing microtome. Numerous antigenic Pick Body-staining were located in neocortical and limbic cortical layers 2, 3, and 5. It were immunoactive to ALZ 50, and antibodies against PHF and ubiquitin. In addition, numerous ALZ-50 positive neurones were seen in the hippocampus. Double immunostaining with ALZ 50 and anti-PHF showed colocalization in many PB. Strikingly, PHF immunostaining revealed extensive neuritic degeneration in neocortex and hippocampal complex similar to that observed primarily in AD. Within the hippocampus, not only were numerous PHF positive PB seen in the dentate granule cell layer, but also extensive neuritic PHF staining was found within the hilar region of the dentate gyrus. An inmunohistochemical study like this study suggests that neuritic degeneration primarily associated with AD occurs in a subpopulation of Pick’s Disease. (Supported by A090466 and AG10161.)

580.7

Immune responses to the GABA synthetic enzyme glutamate decarboxylase (GAD) may play an important role in the development of insulin-dependent diabetes mellitus (IDDM), Stiff-man syndrome, and IDDM-autoimmune neuropathy. Years before the clinical onset of IDDM, we can detect autoantibodies to one or both forms of GAD (GAD65 and GAD67), synthesized from their respective cDNAs in recombinant bacteria. Although autoantibodies to GAD generally decline after IDDM onset (with the loss of the antigen containing β-cells), IDDM patients who develop acute neuropathies have high titer of these antibodies. These results suggest the continued stimulation of the immune system by GAD released from damaged peripheral nerves.

To study the role of GAD in the pathogenesis of IDDM, we have examined the longevity of T-cell responses to known IDDM autoantigens in a murine model of IDDM, the NOD mouse. Of the 55 autoantigens tested (GAD65, GAD67, glutamic acid decarboxylase), the T-cell response to GAD is the earliest, suggesting that GAD may be a primary autoantigen in IDDM. (Supported by grants from NIH, JDF and the ADA)

580.8

Many biochemical and functional alterations have been observed in a number of diabetic rat organs, such as, among others, the variations in the content of carnitines related to the metabolism of glucose alternative substrates. However, the brain as the only one organ that maintains a normal glucose metabolism in diabetes seems to contradict this relation, in fact, in diabetic rats brain carnitine levels are dramatically reduced (-32%). Recently, acetylt-L-carnitine (ALCAR) has shown to be beneficial in modifying biochemical, electrophysiological and motor alterations observed in the diabetic rat. Thence, an experiment was set to verify whether ALCAR was able to compensate for the loss of brain carnitines. Male Sprague-Dawley rats, weighing 300 g, either normal or diabetic (diabetes was induced with 50mg/kg i.v. streptozotocine) were used. Diabetic rats were treated daily for 14 weeks with i.p. 10 mg/kg vehicle or 50mg/10mg/kg ALCAR. At the end of treatment, the rats were sacrificed and the cerebral cortex was extracted. No differences were observed in all three main classes of carnitines. They also indicate that ALCAR corrects both qualitatively and quantitatively the reduction in brain carnitines and are in accordance with the beneficial effects exerted by ALCAR in diabetes-induced neuropathies.

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580.9

Sciatic nerves of diabetic BB/Wor control female and female diabetic BB/Wor rats were analyzed by immuno-precipitation to determine their content of the a1 and a2 isoforms of the catalytic subunit of Na,K-ATPase. Immunobeads were prepared with the McAb and McAb antibodies developed by Swadner and associates (JBC 264:3871, 1989) and employed according to the protocol of Fambrough and Bayne (JBC 258: 3523, 1983) with modifications. The eluted antigens were separated by SDS-PAGE, silver-stained, and quantified with the 2D-ANALYST program. Only a1 was found in detectable quantities. The average amount in control nerves was 1.6 pmol per mg protein (microsomal pellet) vs 1.12 in nerves of age-matched rats after 16 weeks of insulin-dependent diabetes, corresponding to a 30% reduction. The generally higher levels of Na pump in axons as compared to glia, suggest a neural deficit. (Supported by JDF grant 190919 and NIGMS grant SS RR 08224).

580.10

Stress-induced diabetic and age-matched saline-injected control rats were perfused 3 and 7 days after 2, 3 and 7 days post-induction. At 3-7 days, degenerating axon terminals showed a vacuolated electron lucient cytoplasm and clustering of small spherical agranular vesicles. The affected dendrites were also vacuolated electron lucient cytoplasm containing swollen mitochondria and disrupted neurofilaments. At 1-6 months, degenerating dendrites were hypertrophied and contained numerous vacuoles, tubulovesicular elements and unidentified electron dense granules. These degenerating dendrites were post synaptic to either normal or affected axon terminals. Some myelinated axons showed vacuolation and displacement of their axonplasm. Degenerating somata contained a condensed nucleus and numerous vacuoles of various sizes, probably formed from diluted RER. At 9-12 months, degenerating dendrites showed an electron dense cytoplasm containing swollen mitochondria and dilated RER. Degenerating axon terminals were characterized by an electron dense or lucent cytoplasm containing swollen mitochondria and small spherical agranular vesicles. These degenerating axon terminals were presynaptic to either normal or affected dendrites. A few myelinated axons also showed electron dense degeneration. However, the somata appeared to be normal. We suggest that various metabolic disorders in the experimentally-induced diabetic rats may have contributed to these structural changes which may affect the synthesis of neurotransmitters in the paraventricular nucleus of the rat.
TREATMENT OF EXPERIMENTAL DIABETIC NEUROPATHY WITH ACETYL-1-CARNITINE
Dept. of Medical Pharmacol., +Dept. of Physiol. and Biochem., Univ. of Milan; *Inst. for Res. on Senescence, Pomezia, Italy

We have previously shown that acetyl-1-carnitine (ALCAR) treatment prevents the decline of nerve conduction velocity as well as the establishment of autonomic neuropathy in diabetic rats. We now report that in the sciatic nerve of alloxan treated rats a 50% reduction of substance P (SP) accumulation occurs at a ligature point, indicating a marked impairment of both anterograde and retrograde peptide transport. In the same animals we have also examined at the electron microscope the basal lamina of blood vessels and axons of the sciatic nerve. In diabetic animals the basal lamina is thicker than normal and made up of multiple layers. In ALCAR treated diabetic rats the basal lamina is normal and the SP axonal transport is at control values as well. These data further support the potential value of ALCAR treatment in diabetic neuropathy.


Brain amyloid deposition is a pathologic hallmark of Gerstmann-Sträussler-Scheinker disease (GSS). The major component of amyloid fibrils isolated from patients of the Indiana family with GSS is an 11 kDa fragment of PrP spanning residues 58 to *150. This family carries a missense mutation of PRNP gene, causing a codon substitution at position 198. We studied fibrillogenesis in vitro using synthetic peptides homologous to residues 57-64, 106-126, 127-147, 181-205 wild-type and 181-205 with mutant Ser 198. Peptide 57-64, corresponding to the repetitive octapeptide of the PrP molecule, did not produce fibrils. Peptides 106-126 and 127-147 readily formed 8 nm fibrils; the latter also aggregated into paired helical-like filaments. Peptide 181-205 showed only a moderate tendency to produce fibrils, which was not influenced by the 198 mutation. The fibrillogenesis of this PrP mutant was prevented by addition of the absence of this PrP segment in amyloid fibrils formed by patients of the Indiana kindred with GSS. Thus it appears that PrP codon 198 mutation does not directly influence amyloid fibril formation.


In several neurodegenerative diseases, which have an accompanying panning, including Alzheimer's disease (AD), Down's syndrome (DS), Pick's disease and Parkinson's disease (PD), there is a significant loss of locus coeruleus (LC) noradrenergic neurons. In AD, DS and PD we have shown disease-specific patterns of LC cell loss across the rostral-caudal extent of the nucleus. The present study sought to determine whether there are changes in the distribution of LC cells across the rostral-caudal extent of the nucleus in cases of frontal lobe dementia (FLD). Six FLD cases (mean age - 85 years; mean disease duration - 11-17 years), were compared with seven normal controls (mean age - 67 years). The normal LC spans a rostral-caudal distance of 11-14 mm, and contains 16,773 ± 1,133 cells (mean ± SEM) on one side of the brain. There was no significant difference in the length or number of LC cells in the FLD cases. These data indicate that FLD is like multi-infarc dementia in that there is no degeneration of the LC neurons. Supported by AG-08013.
520.17
BIO Maine DEFICITS OBSERVED IN THE VARIOUS STAGES OF THIOCTAINE INDUCED HEPATIC ENSPHALOPATHY IN MALE RATS. B.S. Morton, J.P. McConnel and J.J. Rodriguez-Saiz, Dept. of Cell Biology and Anatomy and Radiology, University of Nebraska Medical Center, Omaha, NE 68194. We have described (in press) a new model of hepatic encephalopathy (HE) in male Sprague-Dawley rats which is characterized by 2 stages. In the initial stage, the rats are fed a diet high in protein. In the second stage, the HE syndrome is induced by the administration of thioctaime (250 mg/kg, intraperitoneally) daily for 7 days. The HE syndrome was classified into 4 stages based on the time course of the encephalopathy. The results demonstrate a decrease in biochemical responses of thioctaime-treated rats as compared to saline treated controls. In stages I and II, the rats exhibit decreased behavior and a gradual increase in liver enzymes. Upon reaching stage III, the behavioral impairment and the elevated liver enzymes are greatly increased. This difference continued until the time animals reached coma stage IV. Our results provide evidence for a behavioral model for the study of acute HE in animals. This model could provide a source for uncovering factors contributing to the encephalopathy. (Supported in part by the Department of Radiology, UNMC.)

520.18
MITOCOCHONDRAL mRNA ENCODING FOR CYTOCHROME OXIDASE SUBUNITs CHANGE IN RESPONSE TO DISRUPTION OF DOPAMINE. A. Grodin, P. S. Damies, and A. Y. Deutch, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510, and VA Medical Center, West Haven, CT 06516. Recent data have indicated that there is a decrease in mitochondrial complex I activity in tissue from Parkinson’s disease (PD) patients. This appears to result from a change in mitochondrial DNA (mtDNA). We have therefore examined changes in mitochondrial specific cytochrome oxidase (CO) subunit gene expression. Administration of 3-ethylcynoline (3-AP) to rats results in a slow progression of the loss of dopamine (DA) innervation of the dorsal striatal (Cp1); the DA innervations of the medial striatum (Cp2) and nucleus accumbens (NAS) are spared. 3-AP rapidly and completely lesion the climbing fiber innervation of the cerebellum. Both CO I and II mRNAs increased in the Cp1, CO II mRNA progressively increased up to eight days after 3-AP treatment. The mitochondrial specific 165 ribosomal RNA also increased in the Cp1. No changes in CO I or II mRNAs in the Cp2 or NAS were observed. In contrast, 3-AP treatment resulted in a decrease in CO gene expression in the cerebellar vermis, Houk et al. treated for 21 days did not show changes in CO I and II. The present data suggest that DA neurons undergoing degeneration exhibit a compensatory increase in CO gene expression, consistent with the increased physiological activity of these neurons, while neurons that have degenerated (cerebellar climbing fibers) exhibit decreased mitochondrial gene expression. Supported by the National Parkinson Foundation Center, MI-45124, and TG GM-07324.

ISCHEMIA: DRUG TREATMENT I

521.1
Effects of the 21-Aminosteriod Tri-hexyloxy Molydate (U-74006F) on the Eicosanoid Levels in the Gerbil Brain Following Ischemia and Reperfusion. P.K. Andrea*, E.H. Hall, B.M. Taylor, L.M. Sam, and P.F. Sog*, CNS Div. Res. and Hypersia. Div. Res.1 The Upjohn Co., Kalamazoo, MI 49001. The present study measured the early production of eicosanoids in the gerbil brain during early reperfusion, following an insult (either 3 h unilateral carotid occlusion (UCO - model of focal ischemia) or a 10 min bilateral carotid occlusion (BCO - model of global ischemia). Arachidonic acid (AA) metabolites were determined to determine if reperfusion with U-74006F could influence brain synthesis of eicosanoids. In the severe UCO ischemia model, there was an early (5 min) post-reperfusion elevation in brain levels of PGE2, TxB2 and LTC4. In contrast, PGE2 and 6-keto-PGF1α decreased. Pretreatment with known neuroprotective doses of U-74006F did not affect the increase in PGE2 or TxB2, but blunted the rise in LTC4. The decrease in PGE2 and 6-keto-PGF1α was also attenuated. In the less severe BCO model, there was a post-ischemic increase in all of the measured eicosanoids. U-74006F decreased the rise in TxB2 and LTC4, but did not affect the other eicosanoids. The results of these studies suggest U-74006F can selectively decrease TxB2 levels in the less severe model and it selectively inhibits leukotriene synthesis in both models. The inhibition of leukotriene formation is believed to be the manifestation of the lipid antioxidant properties of U-74006F, as lipid peroxides are potent activators of S-Lipoxigenase.

521.2
LAZAROID PROTECTION AGAINST NEUROTOXICITY IN CEREBELLAR GRANULE CELL MODELS. G.J. Pici, J.S. Albus, D.E. Decker, S.E. Buxer and P.F. VonVeigelander, The Upjohn Company, Kalamazoo, MI 49001. Novel in vitro methods for testing lipid peroxidation inhibitors (lazaroids) as protectant of neurotoxic insults in cerebellar granule cells were developed. We showed that these cells are susceptible to injury induced by buthionine sulfoximine (BSO), an inhibitor of γ glutamylcysteine synthetase, and ferrous ammonium sulfate (FAS), an initiator of lipid peroxidation. This study revealed that 30,000 µM BSO after 24 hrs produced significant reduction (~15-60%) of control in cell viability, which corresponded to a decrease in glutathione (GSH) levels of 80% or greater. A BSO resistance model was established as well and it produced 95-98% cell death relative to control. The 21-aminosteroids, U-74006 and U-74005, that were delivered to cells in a micrometion delivery differing effects. U-74006 (0.1-100 µM) was unable to protect in either toxicity model. U-74005 (10 µM) significantly protected across the entire toxic range of BSO. In addition, cells were completely protected in the FAS toxic model at 1 µM. At this concentration, amount of actual drug delivered to cells was ~0.5 mole%. Measurements of GSH in the BSO-treated cells showed no sparing or protection of GSH with drug and GSH declined 35%-90% across the BSO dose range of 1-1000 µM. The ability of U-74005 to protect in these models may be a function of greater antioxidant activity (measured by cyclic voltammetry). It is hoped that these model cells of neurotoxicity will further reveal mechanisms of lazaroid mediated neuronal protection.

521.3
NBOX DOES NOT ALTER THE ISCHEMIA-INDUCED REDUCTION OF KAINATE/AMPA RECEPTOR GENE EXPRESSION IN RATS. W.A. Pulichetti, D.E. Pellegatto, Giampietro, S.R., and B.S. Zulak, Cornell University Medical Center, New York, NY 10021, and A. Einstein Coll. Med., Bronx, NY 10461. We reported (see Pellegatto et al., these proceedings) that transient forebrain ischemia in rats selectively reduces expression of the GluR2 subunit that controls Ca2+ permeability in CA1 hippocampal neurons. This reduction of GluR2 message may be causative to CA1 neuronal injury. In this study we examined whether NBOX altered ischemia-induced changes in kainate/AMPA receptor messenger. Sacrificed animals were subjected to ischemia (10 min of forebrain ischemia were immediately after reperfusion with either vehicle, NBOX (30 mg/kg, i.p.), or M-K801 (2.5 mg/kg, ip)). Twenty-four hours later glutamate receptor gene expression in CA1 hippocampus was examined by in situ hybridization. The GluR2 message in CA1, but not in CA3 or dentate gyrus, was markedly reduced. Message for GluR1 and NMDAR1 were little affected. However, neither NBOX altered ischemia-induced changes in glutamate receptor expression. The neuroprotective response of NBOX may be due to blockade of kainate/AMPA receptors, rather than the of the mechanism causing the downregulation of GluR2 expression.

521.4
GLUTAMINETHANE SULFATE IS NEUROPROTECTIVE TOWARD DELAYED CAI NEURONAL DEATH IN THE GERBIL MODEL OF FOREBRAIN ISCHEMIA. H. Izawa, J. Iseki, and T. Nakada, Neurochem Res Lab, VA Res Service, Marticres, CA 94553 and Dept of Neurology, Univ of Calif, Davis, CA 95616. Glutaminethane sulfonate (GES) is a taurine analogue originally introduced as a competitive transport inhibitor of taurine. Recent studies indicated that GES in brain cortex may function as an additional alkali which in turn protects brain tissue against intracellular lactic acidosis. Indeed, GES has been shown to enhance the survival rate of mice exposed to anoxia. In this study we investigated the protective effects of GES on delayed CAI neuronal death using a gerbil model of forebrain ischemia. Pretreatment with GES (125 mg/kg/day IP for 2 weeks) showed significant neuroprotection on CA1 neurons studied 7 days after 5 minutes bilateral carotid occlusion. The results indicate that intracellular acidosis likely plays a role in mediating the harmful effects of ischemia, perhaps in the early steps of a deleterious cascade leading to excitatory amino-acid excess.
521.5

VOLTAGE SENSITIVE AND RECEPTOR OPERATED CALCICUM CHANNEL DENSITIES IN FOCAL CEREBRAL ISCHEMIA. M.J. Hogan, S. Takizawa, A. Giedda*, A.M. Hakim. Cerebrovascular Research Unit, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

We have measured the number of L-type voltage sensitive calcium channels (VSCC) and N-methyl-d-aspartate (NMDA) receptors in acutely ischemic rat brain using autoradiographic analysis of the L-type VSCC antagonist ²¹H]nimodipine and the competitive NMDA receptor antagonist ¹¹C]CGS-19755. Focal cerebral ischemia was produced in male Sprague-Dawley rats under halothane anesthesia. After four hours of ischemia the rats were decapitated, the brains removed, frozen and sectioned at 20 μm onto glass slides. These were then dipped in incubation baths containing either ²¹H]nimodipine or ¹¹C]CGS-19755. Washed and dried sections were then exposed to tritium sensitive photographic film and regional ligand binding determined autoradiographically. The maximal number of binding sites (Bmax) for nimodipine in ischemic and contralateral non-ischemic frontal cortex were 16.3 ± 1.6 and 24.4 ± 1.1 pmol/g respectively. For the CGS-19755 these values were 217 ± 5 and 228 ± 14 pmol/g. In-vitro ²¹H]nimodipine binding is comparable to in-vivo binding in ischemic tissue only and does not demonstrate focal VSCC activation. This can only be demonstrated in-vivo. Similarly, focal increases in NMDA receptors are not observed in-vitro after 4 hours of ischemia. Since NMDA receptor sites fold more common than VSCC’s they may be more important to calcium influx during ischemia.

521.6

LACK OF PROTECTION BY THE ALPHA-2 ADRENERGIC RECEPTOR AGONIST DEPENDEMIDEMINE IN A FOCAL MODEL OF CEREBRAL ISCHEMIA. C.M. Meier, D. M. Kunis, O.H. Sun, T.Z. Quo, M. Maze, and G.K. Steinhberg. Dept of Neurosurgery and Anesthesia, Stanford University Medical Center, Stanford, CA 94305.

Desmedemidine, a highly selective alpha-2 adrenergic agonist, decreases central sympathetic activity and reduces the anesthetic requirement of halothane. Preliminary studies show that desmedemidine improves the outcome from ischemic injury and thus may have potential therapeutic value. We studied eleven rabbits that underwent a two hour occlusion of the left internal carotid, anterior cerebral, and middle cerebral arteries followed by four hours of reperfusion. Ten minutes after occlusion the animals were treated with either normal saline (n=5) or desmedemidine (n=6) using a computer controlled infusion rate set to maintain a steady state plasma concentration. Halothane concentration was reduced by 50% for drug treated rabbits in order to maintain a comparable level of anesthesia. Preliminary results show that desmedemidine, in the dose given, does not have a neuroprotective effect in this model of focal cerebral ischemia.

521.7


We previously reported that the synthetic omega-conopeptide SNX-111, a blocker of calcium flux through voltage-sensitive neural calcium channels, protected hippocampal CA1 pyramidal cells from ischemic damage in the rat 4-vehicle occlusion model of reversible forebrain ischemia when administered by iv bolus injection either immediately or 1 hour post-occlusion. We now report that (1) 5mg/kg SNX-111 infused IV over 15 minutes at 6 hours post-occlusion significantly decreases necrotic damage (p<0.05: n=4 rats/subjected) to either 15 minutes of forebrain ischemi abdomed by common carotid occlusion (2-V:OC) plus hypotension, and (2) neuroprotection is provided when a single iv bolus injection of 5mg/kg SNX-111 is administered up to 24 hours after the ischemic insult in 4-V:OC (CA1 damage relative to untreated controls: 6 hr = 19%, 12 hr = 26%, 24 hr = 19%). Animals receiving SNX-111 under the latter conditions show comparable levels of protection when sacrificed at 5 or 12 days post-treatment, indicating that there is no simple delay, cell loss after the ischemic insult. These findings (1) provide the first demonstration of neuroprotection in the rat 4-V:OC model by delayed treatment with a neuronal calcium channel blocker and (2) confirm that SNX-111 is neuroprotective in two different rat models of global forebrain ischemia.


521.8

EFFECTS OF DISTINCT w-CONOPEPTIDES ON THE RELEASE OF GLUTAMATE AND GABA IN-VIVO. B. Newcomb and A. Palma. NEUREX Corporation, Menlo Park, CA 94025.

Two synthetic w-conopeptides, SNX-111 and SNX-183, originally isolated from the cone snails, C. magus and C. striatus, respectively, have revealed a diversity of voltage-sensitive neuronal channels (Matsuda et al. Soc. Neurosci. Abstr. 17, 1161, 1991). We used in-vivo dialysis to define the actions of these conopeptides on the release of amino acid transmitters in the dorsal hippocampus of the rat. The release evoked by potassium perfusion was suitable for quantitative comparison across experiments, and release of glutamate and GABA could be blocked with 10μM magnesium. SNX-183 and the structurally similar peptide SNX-230 (M.V.IIc from C. magus; see abstracts by Hilyard, et al. and Gaur, et al.) blocked the release of glutamate and GABA at much lower concentrations than required for SNX-111. The probe concentration of SNX-230 giving 50% of maximal inhibition was 2 μM, as compared to 200μM for SNX-111. Dose response curves for glutamate and GABA were similar in the hippocampus, while GABA release in the thalamus was comparatively resistant to the conopeptides. Chromatographic analysis showed that differences in potency were not due to differential degradation.

521.9

EARLY AND CONSTANT RELEASE OF NIMODIPINE FROM IMPLANTED PELLETS IN CONSCIOUS RATS FOR 24 HOURS. A.J.Patrick, J. St. Lefebvre, and E. Lefebvre. Laval University, Quebec, Canada.

Experimental and clinical studies have shown potential benefit of nimodipine (NP) in cerebral ischemia. It is important to evaluate methodologies for continuous administration of NP that may reduce the complexity of experimental studies. Sprague-Dawley rats were briefly (5 min) anesthetized during pellet implantation and assigned into two groups. Pellets (PE) and Placebo (PL). PE rats (n=14) received either 0.5, 1, 2 or 15 mg pellets and PL rats (n=5) received placebo. Pellets were implanted in the naphe of the neck. Plasma levels of NP were measured at 1, 3, 5, 10, 15 and 24 h. The plasma levels obtained with the 15 mg pellets are known to have a therapeutic effect on brain ischemia using such short duration can be easily achieved within one hour and maintained for 24 h. As shown in the figure, NP levels resulted from 2 and 15 mg pellets did not achieve the same mean body concentration (plasma concentration normalized to body weight) and administration of PE or as did lower doses. These results indicate that pellets may be suitable for further evaluation of NP in experimental cerebral ischemia. Also it is important to emphasize that plasma levels of NP in PE rats were significantly lower in the mean arterial blood pressure (>0.05). (Supported by AHA-NEO F244, National AHA 91015180 and Miles, Inc.)

521.10

EFFECTS OF BERAPROST Na (BPS), A PROSTA CYCLIN ANALOGUE, ON PROGRESSIVE CEREBRAL ATROPHY IN GENILS. S.Endo, Y. Miyayuchi, N. Izumimoto, S. Matsuda and T. Endo, Basic Research Laboratories, Toray Industries Inc., Kamakura 248, Japan.

We have already reported that BPS, a new procyclin derivative, has protective effects against various ischemic models. In the present study, we investigated the effects of BPS on progressive cerebral atrophy in gerbils. The unilateral carotid artery of gerbil was repeatedly occluded. At the first ischemic procedure, we selected the symptomatic animals according to their stroke index score (K.Ohno, Brain Res. 297: 151, 1984), its score greater than 10. The selected animals were administered BPS at a dose of 1 to 100 μg/kg, p.o. twice a day for 4 weeks. We measured area ratio of ischemic hemisphere to opposite one with image processing system (Olympus, Japan). In the cerebral cortex of ischemic hemisphere, neuronal loss, acidophilic neurons and progressive atrophy were observed with increasing time of reperfusion after ischemic insult. On 4 weeks after the first ischemic episode, area ratio was approximately 90% in the control animals. BPS was found to inhibit the atrophy of ischemic hemisphere dose-dependently. Our results suggest that procyclin has a protective effect on the ischemia induced progressive atrophy.

Activation of NMDA receptors results in a Ca2+-dependent release of Nitric Oxide (NO). Thus a role for NO has been postulated in NMDA receptor-mediated excitotoxicity. Sustained NMDA receptor activation results in neutropenia and the subsequent accumulation of intracellular Ca2+ is thought to be responsible for the selective pattern of delayed neuronal death seen in the CA1 region of the hippocampus. An attempt to offset the neurodegenerative process was made by treating animals with N-[2-thioalkyl]arginine (NAG), a competitive inhibitor of NO synthase (NOS). NAG was administered for 4 days prior to a single 5 min. period of global forebrain ischaemia. Induction of occlusion of both common carotid arteries. NAG was also administered throughout the survival period. Four days after reperfusion animals were sacrificed and the extent of neuronal damage determined histologically. No protection against ischaemic damage was observed of either dose used. To verify the degree of enzyme inhibition observed by NAG, NOS activity was assessed in crude enzyme extracts of forebrain by measuring the conversion of (H4)-L-arginine to (H4)-citrulline. At the doses used NAG inhibited NOS activity by 80% when compared to saline treated animals. Contrary to in vitro studies on hippocampal slice preparations our data suggest that inhibition of NOS activity does not have a neuroprotective role in ischaemia.

522.2 BLOCKADE OF NITRIC OXIDE SYNTHETASE BY NITRO-ARGININE FAILED TO PROTECT THE BRAIN AGAINST TRANSIENT FOCAL ISCHEMIA IN TWO RAT MCA MODELS: D. Xiong*, Z.G. Huang, S.Z. Gertler and A.M. Buchan. Neurosciences Unit, Ottawa Civic Hospital, Ottawa, Canada, K1Y 4E9.

NMDA and AMPA receptor antagonists have been shown to reduce cerebral injury following focal ischemia. A positive link between nitric oxide (NO) production and glutamate receptor activation on cyclic GMP has been proposed through in vitro culture studies. The possible effectiveness of NO synthetase inhibition by nitro-arginine in treating ischemic brain injury associated with an over-stimulation of glutamate receptors has been suggested. We studied this by using two focal stroke models. Transient focal ischemia was achieved by temporary clamping of the right middle cerebral artery for 2 hours followed by 22 hours of recovery in both Wistar (n=14) and SHR (n=21). Treatment with either saline (1 ml) or nitro-arginine (2 or 10 mg/kg) was given IV 40 minutes prior to ischemia. Regional cerebral blood flow was measured at the time of drug infusion, the onset of ischemia, arterial reperfusion, and sacrifice (24 hrs.). The volume of cortical infarction was then quantitated with frozen-sectioned brain slices. One-way analysis of variance was used.

Wistar: Saline 19±8±5 Drug (10 mg/kg) 19±3±5 SHR: Saline 16±4±9 Drug (2 mg/kg) 15±1±2

Drug (10 mg/kg) 14±5±1 NS

Pre-treatment with nitro-arginine, unlike glutamate antagonists, failed to protect the brain against transient focal ischemia.

522.3 FAILURE TO PROTECT HIPPOCAPMAL CA1 NEURONS BY NITRIC OXIDASE SYNTHETASE INHIBITION. H.U. Gertler, S.Z. Gertler and A.M. Buchan. Neurosciences Unit, Ottawa Civic Hospital, Ottawa, Canada, K1Y 4E9.

Nitric oxide, a free radical, has recently been postulated to play an important role in a number of biological processes. Its proposed role as a mediator of ischemia-induced neuronal cytotoxicity was studied in a 4-vessel occlusion (4-VO) rat model which induces selective neuronal death. We have previously shown that this process is linked to AMPA, but not NMDA, glutamate receptors. Adult male Wistar rats were subjected to 10 min. of severe transient forebrain ischemia using a modified 4-VO model. Nitro-arginine (10 mg/kg), a potent nitric oxide synthetase inhibitor, was infused intravenously 1 hour prior to ischemia. Saline-injected animals served as controls. Criteria rats were sacrificed 7 days later. The number of damaged CA1 neurons was counted and calculated as a percentage of the total number of CA1 neurons. A Mann-Whitney U test was employed.

% of Hippocampal CA1 Injury

Group (%) Mean±SD
Saline (8) 77.6±10.4
Arginine (8) 78.4±11.3 NS

In this experiment, inhibition of nitric oxide synthetase failed to change the outcome of hippocampal CA1 neurons from ischemic injury.


Nitric oxide (NO) has recently been implicated as a key mediator of N-methyl-D-aspartate (NMDA) receptor-associated glutamate neurotoxicity in primary neuronal cell culture (1). We have also demonstrated that NMDA antagonists are potent neuroprotective in several adult rat models of focal ischemia. We sought to obtain evidence for NO production in cerebral ischemia by examining brain levels of "markers" of NO production (NO2-, NO3- and cGMP) following varying periods of focal ischemia. Adult Wistar rats were subjected to bilateral carotid ligations followed by cerebral ischemia (MCA) such that reliably produces a neuronal and subsequently a histologic damage to the contralateral hemisphere. We find a marked increase in NO synthetase activity in brain. We have previously shown that the number of damaged CA1 neurons was counted and calculated as a percentage of the total number of CA1 neurons. A Mann-Whitney U test was employed.

% of Hippocampal CA1 Injury

Group (%) Mean±SD
Saline (8) 77.6±10.4
Arginine (8) 78.4±11.3 NS

In this experiment, inhibition of nitric oxide synthetase failed to change the outcome of hippocampal CA1 neurons from ischemic injury.


Treatment of cultured rat neurons with novel kappa opioids derived from the arylacetamide series, including PD117302 (PD) and CI-977, provides significant protection from glutamate-induced cell injury. Systemic treatment with these compounds has also been reported to provide neuroprotection from focal ischemia and to improve functional outcome following global cerebral ischemia in rats. We now report neuroprotection with PD to rats subjected to 15 minutes of severe forebrain ischemia (4-vessel occlusion). PD (0.2, 1, and 5 mg/kg, ip.) was given at reperfusion and at 2, 4, and 6 h post occlusion. Animals were perfusion fixed 2 h later. Ischemic injury was quantified in the CA1 of the hippocampus. In saline-treated rats ischemia caused extensive and consistent loss of pyramidal CA1 neurons. PD produced significant protection of CA1 neurons which was dose-dependent and nearly complete at the 5 mg/kg dose. Thus, kappa opioids from this series may be therapeutically effective in the treatment of ischemic neuronal injury.

522.6 EFFECT OF CI-977 ON ISCHEMIC BRAIN DAMAGE AND CEREBRAL BLOOD FLOW AFTER MIDDLE CEREBRAL ARTERY (MCA) OCCLUSION IN THE RAT. K.B. Mackay, K. Kasumoto, D.J. Graham & J. McCulloch*, Wellcome Neuroscience Group, University of Glasgow, G61 1QH, U.K.

Selective kappa agonists (such as CI-977) are putatively beneficial in some animal models of cerebral ischemia. The mechanism for these effects is unclear. We have investigated the effect of CI-977 on ischemic brain damage and cerebral blood flow (CBF) in a rat model of focal cerebral ischemia. The left MCA was permanently occluded under halothane anesthesia in male Sprague-Dawley rats. Four h later, animals were sacrificed by cardiac perfusion with phosphate buffered saline at 2, 4, and 6 h post occlusion. Cerebral blood flow (CBF) was measured 30 min post-occlusion using [14C]-iodoantipyrine autoradiography. Pre-administration of CI-977 (0.3mg/kg, s.c.) significantly reduced the volume of ischemia brain damage in cerebral hemisphere (by 27%) and cerebral cortex (by 32%) when compared to controls. CI-977 had no significant effect on either local CBF in any of the 24 regions examined or on the cumulative CBF volume in the hemispheres ipsilateral and contralateral to the ischemic MCA territory compared to controls. These results indicate that the neuroprotective effects of CI-977 following permanent MCA occlusion in the rat cannot be attributed to improved blood flow to ischemic tissue.
522.7

US4948 REDUCES THE SEVERITY OF TISSUE DAMAGE IN A RABBIT MODEL OF FOCAL CEREBRAL ISCHEMIA
M.A. Widmayer, J.L. Browning and D.S. Baskin*. Dept. of Neurosurgery, Baylor College of Medicine and Research Service, Houston VA, Houston, TX 77030.

Following successful investigation of kappa agonist treatment of focal cerebral ischemia in the cat, we have broadened our research to include a more readily available and less costly species. Fourteen male, NZ white rabbits underwent occlusion of the right middle cerebral, anterior cerebral, and internal carotid arteries, beginning at their trifurcation and extending 1 mm distally along each vessel. Six hours later half of the rabbits received an injection of 0.5 mg/kg US4948 (US50) and s.c. osmotic pump infusion of 0.4 mg/hr US50, and half received saline (SAL.). Rabbits were sacrificed on the eighth post-op day and their brains stained with TTC. Abnormally stained tissue was categorized into infarct (colorless) and ischemic (lightly stained). Generally, abnormally stained tissue comprised 45% of brain tissue regardless of treatment. However, compared to saline treated rabbits, rabbits treated with the kappa agonist US50 had smaller infarcts (mean ± sem = 35.3 ± 5.9% vs. 18.1 ± 4.5%; p = 0.05) and larger areas of ischemic tissue (12.4 ± 3.6% vs. 24.1 ± 3.7%; p < 0.05) than SAL-treated rabbits.

Additional untreated rabbits were utilized to support the functionality of the infarcted tissue. Transplantation tests showed promise in a species less costly and more available than cats.

522.9

THE SELECTIVE GLYCINE ANTAGONIST 7-CHLORO-THIO-

Ischemic brain injury is mediated, at least in part, by the release of glutamate and other excitatory amino acids and neurotransmitters activating a receptor gated Ca channel. Occupation of the strychnine insensitive glycine potentiating site is required for opening of the NMDA receptor gated Ca channel. Blockade of glycine binding might therefore be expected to attenuate neuronal injury in brain.

We examined the effect of the selective glycine antagonist 7-chloro-thio-kynurenic acid (20mg/kg iv) given either 5min prior to or 15min or 60min after permanent middle cerebral artery occlusion in rats (n=6 in each treated group with n=9 saline controls). Stroke size was measured by computerized image analysis on serial 20μm cresyl violet stained sections 24 hrs following MCA occlusion. Infarct volume was smaller (p<0.05) in the 5 min pretreatment group (13.1 ± 2.3 mm3) compared to controls (16.8 ± 2.3 mm3). 7-chloro-thio-kynurenic acid (20mg/kg 30min pretreatment), which does not bind to the glycine site has similar antioxidant effects as 7-chloro-kynurenic acid, was not effective (18.3 ± 16.2 mm3).

These data support a potential role for glycine site antagonists in the treatment of stroke.

522.11


As an in vitro model of ischemic damage, cortical neurons were challenged with glutamate (0.5 and 10mM), and the levels of released LDH [lactate dehydrogenase] were monitored at 1hr, 2, 4, and 7 days. Analysis of neuronal degeneration and membrane damage were also studied to further elucidate the mechanisms underlying this lipid's neuroprotective effects. Neuronal cortical cultures derived from 15 day embryos were exposed to 152 and 154 blastic membranes. At 7 days post-Mt.1 treated groups were exposed to glutamate (0.5 or 10mM) for 30min. Parallel cultures were treated with GM1: either a 0min pre-

trials or 1 hr, 1, 2, 3, 4 and 5 days post-treatment with 80μM of GM1. Exposure to 10mM glutamate caused a 70-80% increase in LDH release at 1-4htrs. Pre-treatment with GM1 reduced the release to 50-60% while post treatment reduced the LDH release to 45%. The membrane structure changes observed by the GM1-treated groups were undetectable on the LDH release data. At 7 days only post-GM1 treated cultures showed significant structural integrity as evidenced by continuous staining of GM1 along the perikaryo and processes.

These data further support our hypothesis that GM1 treatment (both pre- and post) reduces plasma membrane damage.

This work was supported in part by a grant from the Fidia Research Foundation.

522.8

EFFECT OF OPIATE ANTAGONISTS ON HYPOXIA AND CEREBRAL ISCHEMIA IN THE RAT. G. Delbarre*, B. Delbarre and F. Calino, Faculté de Médecine, 37000, Tours, France.

In human and animal, the opiates have been shown to have significant physiologic effects on respiratory system and to be implicated in hypoxia and cerebral ischemia.

To determine the role of opiate in these mechanisms, we have studied opiate antagonists (naltrexone) on rat, and, on cerebral ischemia by unilateral carotid ligation in gerbil (Delbarre, G., Stroke, 19:126, 1988).

Naloxone (0.5 mg/kg, Per Os) significantly antagonizes the increase of reaction time of flic tests induced by hypoxia (p < 0.001). Naltrexone (2.5 mg/kg, Per Os) and WIN 44,441, a kappa antagonist (5 mg/kg, Per Os), significantly improve the Stroke Index in gerbil cerebral ischemia (p < 0.05).

Percentage of protection

Tail flick after hypoxia

Naloxone (0.5 mg/kg) 54.93 ***

Ischemia

- WIN 44,441 (5 mg/kg) 51.68 *
- Naltrexone (2.5 mg/kg) 39.71 *

Unpaired Student t test versus control -p < 0.05 *-p < 0.001 ***

These results demonstrate that opiate antagonists might play a therapeutic role in hypoxia and cerebral ischemia.

522.10


Previous experiments have shown that a series of thio-derivatives of kynurenic acid (KYN) displaced β-glycine (GLY) from its strychnine insensitive binding sites in cortical membranes (J. Pharmacol. Exp. Ther. 227, 1991) and antagonized L-GLU-induced neuronal death in cerebellar granule cells in culture (Braz. J. Pharmacol. in press). Among the thio-KYN derivatives the most potent were 7-thio-kynurenamide (its ICSO in displacing β-GLY was 0.4 μM) and 5,7-dichlorothio-KYN (its ICSO was 0.6 μM). Other compounds of the series were less potent and none of the tested molecules (up to 100 μM and displaced β-CPG, thio-KYN or β-CPG) from the respective recognition sites in cortical membranes, suggesting that these kynurenate derivatives were selective GLY antagonists. When 7-thio-kynurenamide or 5,7-

dichlorothio-KYN were compared with the corresponding moieties of KYN it was observed that they had similar potency in displacing β-GLY from cortical membranes, but were 6 to 10 times more potent in preventing L-GLU-

induced neuronal death. In an attempt to explain this particular effectiveness against L-GLU-toxicity we studied 7Cl-KYN, 5,7-ClthioKYN and the corresponding moieties of KYN. A series of thio-KYN derivatives with lipid peroxidation inhibitors in a simple "in vitro" model (Neurchem. Res. 16, 42, 1991). 7-thiocynonamide and 5,7-thiocynonamide may reduce excitotoxic death by acting both as GLY antagonists and as radical scavengers.

522.12

GENETIC MODEL FOR ISCHEMIA - FEMALE STROKE PRONE SPONTA-

Stroke Prone Spontaneously Hypertensive Rats [SHRSP] may provide an advantageous model to study CNS ischemia. This genetic strain of rat develops pathological conditions that are associated with stroke. These include hypertension, arterial thrombosis, fibrinoid necrosis, and hemorrhage. The focus of this study was to detail mortality rates and occurrences of related stroke symptoms using the female SHRSP, and, to assess the neuroprotective effects of GM1 on cerebral ischemia. The female SHRSP rats were randomly assigned to GM1 or saline treatment. Blood pressure was measured 2 days prior to and 7 days post-treatment with 7.5% overnight. At 7.5 weeks all rats were fed Japanese Stroke Prone Rodent Diet (Zeigler Bros., Gardner, PA) and their drinking water was replaced with 1% NaCl. Chronic GM1 treatment (10mg/kg i.m. daily), Fidia Research Laboratory, began at 8 weeks. Post-treatment activity, sensorimotor and neurological performance on a tail-hang task was assessed 3 times weekly beginning at 10wks. At this time the rats were observed daily for overt stroke symptoms (+10% weight loss, +10% appetite loss, +10% motoric movement. The female SHRSP rats exhibited 100% mortality within 92 days of being placed on the diet. GM1 hyperreduced activity over the last 3 weeks prior to the onset of overt stroke symptoms ( +10% symptom severity in latently balanced CNS damage. There was no marked effect on functional measures, weight loss and increased blood pressure. GM1 did not alter the underlying pathologies, but did ameliorate the initial indications of CNS damage. The use and inclusion of fe-

male SHRSP in ischemia research may be helpful to better detail the patholo-
gy and treatment of stroke in patients with similar underlying pathologies.

Supported by a grant from the Fidia Research Foundation.
522.13
GALANTAMINE TREATMENT FAILS TO ATTENUATE COGNITIVE DEFICITS FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA. P. A. Forget, P. W. Parsons, J. J. Briggs, R. S. C. Holloway, Department of Psychology and Gerontology Program, Slippery Rock University, Slippery Rock, PA 16057 and Grove City College, Grove City, PA 16127.

Systemic administration of galantamine has been shown to have significant beneficial effects on the central nervous system following various forms of insult. In the present study, adult male rats were subjected to bilateral common carotid occlusion for ten minutes while anesthetized with sodium pentobarbital. Animals were treated at 10 min after occlusion and again at 24 hrs post-surgery with either GM1 galantamine (20 mg/kg i.p.) or vehicle-alone (control). A third group of nonoperated controls was included in the behavioral analyses. Animals were permitted two weeks to recover and trained daily in an eight-arm radial maze for four weeks. Analysis of behavioral data revealed that galantamine- and vehicle-treated occluded animals made significantly more errors over the last week of training than did unoperated control animals. Differences in working memory errors were not statistically significant.

522.15
MEOSI FAILED TO REDUCE INFARCT SIZE IN THROMBOTIC MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS. H. Yao, M. D. Ginsberg*.

In animal models of focal cerebral ischemia, almost all studies have demonstrated significant reductions of infarct volume by the noncompetitive NMDA antagonist memantine. However, this drug failed to attenuate focal ischemic insult in our present study. Male Sprague-Dawley rats were randomly assigned to receive either memantine (1 mg/kg) or saline ip 30 min before focal permanent middle cerebral artery occlusion (tMCAO). Irradiation with an argon laser-activated 562 nm dye laser after rose bengal (20 mg/kg) iv was used to cause thrombotic tMCAO. The ipsilateral common carotid artery was permanently occluded and contralateral carotid artery for one hour. In exp. 1, head temperature was controlled at 36°C. Three days after tMCAO, brains were perfused and stained with H&E. Cortical infarct volume was 92±9 (mean±SD) mm3 in treated group, which was not different from 84±60 in control group. In exp. 2, head temperature was monitored but not controlled. Mean head temperature before ischemia was 34.4°C and decreased by 1-2°C after tMCAO while rectal temperature was maintained at 37°C. Head temperature was not different between treated and untreated groups, thus excluding the confounding factor of temperature change. Further results of exp. 2 will be presented. It is suggested from results of exp. 1 that memantine may be ineffective in head temperature controlled, thrombotic focal ischemia.

522.17
OSS-19755 IS NEUROPROTECTIVE DURING REPETITIVE ISCHEMIA: THIS EFFECT IS SIGNIFICANTLY ENHANCED WHEN COMBINED WITH HYPOHYPERAEMIA. A. Shukul+, G. Liakos, H. Howlett, R. Hazzag, Dept. of Medicine, Div. of Neurology, Royal University Hospital, Saskatoon, Sask. S7N 0W0

In small animals the damaging effects of repetitive ischemia are cumulative and exceed that of a single similar duration insult. The more severe damage may be the result of delayed release of glutamate, a neurotransmitter at the proximal site of occlusion at the two to five minute delay. In a model of repetitive ischemia, we used 3 minutes of forebrain ischemia and repeated it 3 times at one hour intervals. Damage scores were assessed 2 days after the last insult. At 2 days, OSS-19755 treated animals showed significantly reduced damage in the cerebral cortex and hippocampus (CA1 and CA3), medial geniculate, and the thalamus. At 7 days, mild protection was seen in the CA1 region of the hippocampus and cerebral cortex. The protective effects of OSS-19755 were greater after the first ischemic insult as compared to animals where the medication was started prior to the initial insult. When OSS-19755 was combined with mild hypothermia, the effects of repetitive ischemia were completely abolished in all but one gerbil. The use of NMDA receptor blockers may protect the brain in repetitive ischemia and this effect is significantly enhanced when combined with mild hypothermia.

522.14
DELAY OF ANOXIC DEPOLARIZATION BY CREATINE, SPHINGOSINE DERIVATIVES, OR MANNIOTOL. M. Balestrino†, I. Cojgliolo*, G. Lunardi*, A. Leon*, S. Maggiardini, Dept. of Psychology, University of Genova, Italy, and Freda Research Labs, Abano Terme, Italy.

Delay of anoxia on the central nervous system (AD) protects neurons from subsequent death (Stroke 21:1111, 179-183, 1990). In the region of Cl both hippocampal slices the following AD latencies were found (seconds, mean±SD): control, 51±9 (N=9); creatine (25mM), 152±40 (N=4, p<0.03); Ligand (138±35); sphingosine derivatives from Fidia; (5mM), 67±15 (N=7) and 85±27 (N=5), respectively (p<0.03 and p<0.01). Additional experiments with “core-skimming” design using mannitol (100mM) increased AD latency (25±9%), compared to paired controls (N=3, p<0.05). The effect of creatine (which delays ATP depletion during hypoxia: J. Physiol. 325: 51-65, 1982) supports the hypothesis (Soc. Neurosci. Abstr. 16: 277, 1990) that block of (Na+, K+)ATPase is the determining factor in AD. The efficacy of Ligand, PFK2 and mannitol suggests a role for both protein kinase C translocation and cell swelling in the delay of AD, and, possibly, in neuronal protection.

522.16
MK-801 PROTECTS IN A MODEL OF REPEATED EPISODES OF BRIEF NONTHROMBOTIC FOREBRAIN ISCHEMIA IN RATS. R. Leib, W. D. Kandel, G. Kruegel, B. Rustad, H. M. Sales, M. E. Gilseng. Cerebral Vascular Disease Research Center, Dept. Neurology, Univ. of Miami School of Medicine, Miami, FL 33101.

We determined whether the non-competitive NMDA receptor antagonist, MK-801 (2mg/kg), would protect the brain if treatment was initiated after the first ischemic insult. Anesthetized rats were subjected to three 5-min periods of global forebrain ischemia by bilateral carotid occlusion (tMCAO) separated by 1-hr intervals. Ischemic brain temperature was maintained at 37°C. MK-801 (5mg) or water (5ml) was injected ip 45 min following the end of the first ischemic insult. Rats were perfusion-fixed at 7 days and regional ischemic cell change (ICC) assessed using a 5-point scale. Serum ICC was documented throughout the CA1 hippocampus, dorsolateral striatum, cerebral cortex and ventrolateral thalamus of non-treated rats. In treated rats, significant protection was documented in all brain regions (p<0.01, Kruskal-Wallis 1-way analysis by ranks). For example, in the ventrolateral thalamus, MK-801 treatment dramatically reduced damage from 2.6±0.4 (mean±SD) to 0.10±0.2. These findings indicate that excitotoxic mechanisms are involved in ischemic damage produced by repeated ischemic insults and that significant protection can be demonstrated with MK-801 even when treatment is initiated after the first insult.

522.18
THE NEUROBEHAVIORAL AND MORPHOLOGICAL PROTECTIVE EFFECTS OF CBST-19755 (AN NMDA RECEPTOR BLOCKER) IN AN ANIMAL MODEL OF ISCHEMIA. T.B. Wishart , D. Liakos, A. Shukul, R. Najjar, J. Kalra, W. Howlett, Depts. of Psychology, Medicine (Neurology) and Pathology, University of Saskatchewan, Saskatoon, Sask. Canada S7N 0W0.

CBST-19755 alone or in combination with mild hypothermia results in significant neuronal protection in an animal model of repetitive cerebral ischemia (25 min ischemia/2 min reperfusion period of one hour, gerbil with ischemia + CBST, and gerbils with ischemia + CBST + hypothermia). Neural damage was assessed by examining sections of silver-stained brain tissue. Animals received 5 trials/day for 7 days in a Morris water maze. Gerbils subjected to repetitive ischemia had widespread neural damage which was significant and sufficient to learn the required response than were animals in all other groups. CBST or CBST + hypothermia had neuroprotective effects in thalamus, striatum, and the hippocampus. There were no behavioral differences between these groups and normal controls. CBST appears to be able to functionally protect animals from the behaviorally disruptive effects of cerebral ischemia.
522.19 ATTENUATION OF ISCHEMIC BRAIN INJURY BY THE NOVEL COMPEITIVE NMDA ANTAGONIST MDL 100,453. 1BH Zielinski, 2BM Baron, 3JP Whitten. Marion Merrell Dow Research Institute, 1Kansas City, MO, 64134, 2Cincinnati, OH, 45215.

The excitatory amino acids glutamate and aspartate have been implicated in the delayed injury to brain tissue after cerebral ischemia through actions mediated by the N-methyl-D-aspartate (NMDA) receptor. The ability of the competitive NMDA antagonist MDL 100,453 to attenuate ischemic injury in a model of focal cerebral ischemia when administered either prior to, or after ischemia induction in spontaneously hypertensive rats was investigated. MDL 100,453 was administered via continuous intravenous infusion with Aza osmotc minigmps over a 24 hour time period starting either 30 minutes prior to (30 mg/kg/24 hr) or 30 minutes after (100 mg/kg/24 hr) transient permanent occlusion of the right common carotid and middle cerebral arteries. MDL 100,453 was found to significantly reduce the volume of the resultant brain injury. The volume (mean ± s.d.) of infarcted brain was 174 ± 27 and 172 ± 15 mm³, in saline treated control animals after pre- or post-occlusion administration, respectively, compared to 110 ± 21 and 124 ± 25 mm³, respectively, in MDL 100,453 treated animals. The amount of protection seen was equivalent to that afforded by the prototype competitive NMDA antagonist CPP (91 ±11 and 119 ± 15 mm³ after pre- and post-MAO administration, respectively). These experiments therefore suggest that MDL 100,453 reduces ischemic damage and may be useful in the treatment of human stroke.

523.1 HYPOGLYCEMIA OR INHIBITION OF GLUCOSE UPTAKE RESULTS IN A LACTATE PREVENTABLE INCREASE IN ADENOSINE AND DEPRESSION OF SYNAPTIC TRANSMISSION IN RAT HIPPOCAMPAL SLICES. L.C. Fouquet, K. Funderburk, A. Davis, 1G. Morell. College of Medicine, Department of Physiology, Texas Tech Health Sciences Center. Lubbock, TX 79430.

The effect of glucose deprivation on adenosine levels and on synaptic transmission was investigated in rat hippocampal slices. Adenosine levels were measured, using absorbance HPLC, in aliquots taken from static chambers of 2 ml volume each containing 4 hippocampal slices. Slices were kept on a net at an interface between the physiologic medium and the humidified atmosphere at a temp of 33-34°C. Electrophysiologic measurements were made from slices under identical conditions.

Incubation of hippocampal slices in either glucose-free medium or in the presence of the glucose transport inhibitor cytochalasin B (50 µM) increased bath adenosine levels and depressed the extracellularly recorded synaptic potential or population spike. The addition of lactate (10 mM), a precursor for mitochondrial ATP generation, prevented the elevation in adenosine and the depression of the population spike. These results indicate that the neuroinhibitory modulator adenosine is elevated during glucose deprivation and contributes to the hypoglycemic depression of synaptic transmission. The rise in adenosine during glucose deprivation can be prevented by providing substrate for mitochondrial ATP synthesis. It is hypothesized that glucose transport inhibition may be a relevant neuroprotective strategy during cerebral ischemia as it may reduce lactic acid accumulation and help maintain elevated levels of the endogenous neuroprotective adenosine.

523.2 HYPERGLYCEMIC HYPOXIA AND INTRACELLULAR ACIDOSIS ENHANCE THE DEPOLARIZING AFTERPOTENTIAL OF RAT MYELINATED AXONS. P. Grafe, U. Schneider, and A.K.E. Horn. Dept. of Physiology, Univ. of Munich, W-800 München 2, Germany.

The mechanism(s) underlying functional abnormalities of peripheral axons in diabetic neuropathy are not well understood. Here we report that the combination of hyperglycemia and hypoxia, two factors possibly involved in the pathogenesis of diabetic neuropathy, enhances the depolarizing afterpotential (DAP) of myelinated axons. Experiments were performed on isolated rat spinal roots maintained at 36°C in a HEPES-buffered solution containing 2.5 or 25 mM glucose. Electrical stimulation and d-c-stable recordings of compound action potentials were performed in a three-chambered organ bath. Hypoxia was induced by replacing oxygen for 30 min with ultrarare gas (F2O<sub>2</sub> < 2 mmHg). Under normoxic conditions, a single action potential was followed by a DAP (duration about 10 ms). During hypoxia in a solution with 2.5 mM glucose, the DAP disappeared. In contrast, hypoxia in 25 mM glucose strongly enhanced the DAP and increased its duration by up to 40 ms. A similar enhancement in amplitude and duration of the DAP was observed after the axons had been acid-loaded by addition of propionate (20 mM) to the bathing solution (at constant interstitial pH). These results indicate that hyperglycemic hypoxia may change the DAP and, consequently, functional parameters of myelinated axons by an acidosis-induced decrease in interstitial membrane conductance.

523.3 MODERATE HYPERGLYCEMIA WORSENS ACUTE BLOOD-BRAIN BARRIER DAMAGE FOLLOWING BRAIN ISCHEMIA. W.D. Dietrich, O. Almeida, L. Fialky, and M. Rusto. Cerebral Vascular Disease Research Center, Dept. Neurology, Univ. of Miami School of Medicine, Miami, Florida, 33101.

We examined the effects of moderate hyperglycemia on the response of the blood-brain barrier (BBB) to normothermic (37°C) cerebral ischemia. Rats underwent 20 min of 4-vessel occlusion followed by 30 min of postischemic recirculation. Hyperglycemic rats received an i.p. injection of 50% dextrose 15 min prior to the ischemic insult. Normosugard rats were used as an indicator of BBB dysfunction. Following normoglycemic brain ischemia (plasma glucose 112±18 mg/dL) or glucose (PG<sub>2</sub>=34±5 mg/dL) and wide spread BBB disruption throughout the neocortex. Sites of protein leakage included the cerebral cortex, striatum, hippocampus, and cerebellum, including damage to the associated neurons and swollen astrocytes. Blood was detected at sites of BBB damage. Intracellular injury had a significant effect on the vascular and neuronal consequences of hyperglycemic ischemia. Under normothermic ischemic conditions, hyperglycemia worsened the degree of BBB disruption compared to normoglycemia. Increased vascular permeability represents an important pathomechanism by which systemic hyperglycemia may aggravate ischemic outcome.

523.4 DURATION OF ISCHEMIA: EFFECTS ON OUTCOME IN NORMOGLYCEMIC (N) AND HYPERGLYCEMIC (H) RATS. DS Warnes*, MM Todd, Paula Ludwig Neuroanesthesiology Research Group, Department of Anesthesia, University of Iowa, Iowa City, IA 52242.

Acute hyperglycemia, prior to global ischemia, often results in seizures and death. The duration of ischemia necessary to elicit such effects was determined in this experiment. Fasted male Sprague-Dawley rats were anesthetized and prepared for forebrain ischemia (FBI). Prior to FBI, rats received either saline (plasma glucose<112±18 mg/dL) or glucose (PG<sub>2</sub>=34±5 mg/dL). After 4, 8, 12, or 15 min of FBI (bilateral carotid occlusion; MA<sub>2</sub>=30±5 mmHg), rats (n=12) were recovered and observed for seizures, motor function, and survival. Rats surviving 7 days underwent perfusion fixation for neurohistologic analysis. Seizure and mortality rates were compared between N and H groups for each ischemic duration. Significant differences were observed for both variables at <8 min of FBI. Seizure rats continued to increase with increased ischemic durations (<p<0.01) while mortality rates stabilized at 8 mins (p=0.42). Motor scores amongst survivors (7d postischemia) were different with >12 min of FBI. The glycemic state of the rat becomes relevant to neurobehavioral outcome between 4 and 8 mins of global ischemia.

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523.5 EXTRACELLULAR DOPAMINE IN HYPERGLYCIC ISCHEMIA. WA Kofke, RR Gorman, RL Stiller, HE Rose, Dept. of Anes/COM, Neurologic Anes, and Supp. Care Progr., Univ. of Pittsburgh, Pittsburgh, PA 15261

We tested the hypothesis that hyperglycemic (HG) exacer-
baration of ischemic striatal damage is associated with an increased elevation of extracellular dopamine (DA).

METHODS: 16 fed male rats were used. Each rat underwent in-
vasions of striatal microdialysers (OCS) probe and was
covered overnight in soundproof isolation. On 2-day vascular
canula temp probe and EEG elec were inserted. GLC 40% or
54 5% O2 and 2% or 4% CO2, resp. After 1 h of
O2/5NO: 100% O2, 0.5 mg Arfonad was given iv, blood re-
moving to MAP 50mmHg, and both carotids occluded for 12m. Life support continued for 6h. NO preeexp was 2 ul/m.

Dispersed samples were assayed for dopamine by HPLC.

Path in the stratum was graded from 0-5 (0=norm).

RESULTS: Five 40F glc and 2.5 glc rats had severe (grade 4 or 5) striatal damage (p<.05). Baseline (BL) DA was nondetectable. NO and ischemia both increased DA vs. BL (p<.05). DA was increased at 30m, 60m compared control and NO (p<.05). No between group DA differences occurred.

CONCLUSIONS: 1) NO after minor surgery in-
creases striatal DA. 2) HG exacerbation of ischemic
stratal damage in rats is not due to increased
extracellular DA. 

523.6 PHYSIOLOGIC AND HISTOLOGIC EFFECTS OF PHENYTOIN IN A RAT GLOBAL ISCHEMIA MODEL. H. Qi, M. Malicki-Sawicka, F. Hirst, J. G. Newman*, SUNY at Stony Brook, NY and VAMC at Northport, NY, 11794.

Phenytoin is frequently studied in rat models of ischemia and epilepsy. Despite this, the pharmacologic effects of phenytoin in the rat have not been studied in detail. Experiments testing phenytoin in a brain slice model of global ischemia revealed a profound concentration-dependent hypoglycemia, up to 25 mmol glucose as well as a profound hypothermia.

Male Sprague-Dawley rats (175-275g) were injected with 50 to 200 mg/kg i.p. and sacrificed 15 mins after drug experiments. Some rats were initially anesthetized with halothane - NO for arterial catheterization and then given phenytoin i.v. 2 hours later with BP and arterial blood gas monitoring. Several factors - drug and water were studied. Histology was assayed by counting the percentage of normal appearing CA1 neurons remaining in 500x thick hippocampal slices after 5 hours in vitro. Phenytoin, at serum concentrations of 6 to 60 μg/ml, caused a linear increase in serum glucose from 8.5 mmol in saline controls to over 25 mmol. The hypoglycemia is reversed by co-administration of insulin i.m., is present to a least the same degree in Fischer 344 and Wistar strains and independent of animal temperature. For phenytoin also induces hypoglycemia whep pyrrole glycol vehicle does not. There is no concentration-dependent hypoglycemia induced by phenytoin. BP and blood gases were not significantly affected by phenytoin. Phenytoin produces only inconsistent effects in CA1 neuronal survival whether phenytoin is continued in vivo or not. 

The present histologic results, along with our prior results in our thick slice model of ischemia, suggest that phenytoin will be more useful in treating focal than global ischemia.

ISCHEMIA: GENE OR PROTEIN INDUCTION

524.1 THE STRESS RESPONSE IS DEPENDENT ON HYPERTERMIA AFTER BRIEF ISCHEMIA IN THE GERBILL. T. S. Nowak Jr. and S. Suga. Stroke Branch, NINDS, NIH, Bethesda, MD 20892.

Previous studies established 2 min ischemia as a threshold insult for producing several changes in gene expression in gerbil brain. Brief periods of ischemia, associated with accumulation of the heat shock / stress protein, hsp70, in vulnerable CA1 neurons, are also reported to induce tolerance to subsequent more severe insults. In our hands both hsp70 expression and induction of tolerance after brief ischemia have been variable. We now show that increases in hsp70 mRNA correlate with the presence of hyperthermia during early recirculation after 2 min insults. Gerbils were subjected to bilateral carotid artery occlusion under halothane anesthesia. Hsp70 mRNA levels were evaluated by in situ hybridization and quantitative densitometry at 3 h or 24 h. Rectal temperature (Tg) was monitored during recirculation and release of anesthesia (Experiment 1) or anesthesia was continued and Tg was either maintained at 37 °C or elevated to 39-40 °C during 30-90 min recirculation (Experiment 2). In Experiment 1, pronounced hsp70 expression was observed only in gerbils with spontaneous Tg ≥ 39 °C. Hsp70 hybridization in all hippocampal fields was several-fold higher in hypothemic animals at 3 h, and lasting hsp70 hybridization at 24 h was only detected in the hyperthermic group (Experiment 2).

Earlier work identified temperature during early recirculation as an potential variable influencing the course of pathology in CA1 neurons after damaging insults. The present results implicate postischemic temperature as a factor reproducing the transcriptional response to tolerance ischemia that may be of particular relevance to the expression of tolerance after brief challenges. 

524.3 DISTINCT PATTERNS OF HEAT SHOCK PROTEIN INDUCTION FOLLOWING FOREBRAIN ISCHEMIA IN RATS. H. Gagey, S. Graham, S. Suga, P. Weinsten*, and P. Stimp, Dept. of Neurology and Neurosurgery, USC Sch. of Med. SP. (CA 90434)

During global ischemia, the synthesis of various heat shock proteins (HSP), including HSP72, is induced in selectively vulnerable neurons, and occasionally in glial cells, in isolated hippocampus, and in striatum. The significance of HSP72 induction in these cells, however, is unclear.

The results suggest the model of simultaneous cortical occlusion and hypotension in rats for either 30 minutes (30) or 60 minutes (60) after 24 hours. Immunohistochemistry was performed using monoclonal and polyclonal antibodies for HSP72. Sections were also stained with NAE or acid fucoid. In addition, sections from brains were double labeled for HSP72 and glutathione reductase antibodies (GPBAR). The results suggest that HSP72 and induction of NAE, markers for astrocytes and microglia, respectively, 

Mild ischemic insults were associated with peak HSP72 induction in neurons in the cortex and striatum, small columns of HSP72 positive neurons in CA1 hippocampus, and no apparent abnormalities with NAE or acid fucoid. Moderate ischemia resulted in diffuse HSP72 induction in neurons throughout the entire forebrain, with occasional glial cell induction. In NAE stained sections, scattered neuronal neurons were evident in the cortex and hippocampus; CA1-4 hippocampal neurons were acid fucoid positive while the dentate gyrus appeared spared. HSP72 was not found in neuronal neurons when slides were counterstained with HAE. In severe ischemia, HSP72 induction was marked in glial and endothelial cells, with no noted neuronal expression, especially in remote regions (i.e. dentate gyrus). Areas with prominent glial induction corresponded to regions with widespread ischemic necrosis and pale surviving on HAE, and acid fucoid positive cells throughout the hippocampus. Double-labeled sections revealed that both microglia and astrocytes (type 1 and II) express HSP72 in these severely injured brains. 

These results confirm previous findings that these markers expressed in injured neurons that survive ischemia. In addition, severe ischemic injury results in both microglial and astrocyte HSP72 expression in the absence of neuronal expression. Thus HSP72 is a valuable early marker to quantify ischemic injury.

524.4 FOCAL HSP-70 mRNA INDUCTION WITH LITTLE TO NO HISTOLOGIC INJURY IN RAT BRAIN. D.M.O'Brien*, S.S. Glacier, F.A. Webb, Div. of Neurosurg, Univ. of Penn., Phila., PA 19104.

A variety of cell stresses including ischemia have been shown to induce heat shock proteins which may help defend cells against injury. The significance of HSP72 induction in these cells, however, is unclear.

The results suggest that inflammatory cytokines are capable of inducing heat shock proteins in neurons and astrocytes and that they may be important in the induction of the acute phase protective response of CNS cells to injury.

Supported by the research service of the Department of Veterans Affairs.

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524.5  

Ischemic stresses inflict numerous changes on neural cellular elements. One such consequence is the production of several classes of stress-response proteins, or heat-shock proteins (HSPs). Both in-vitro and in-vivo models have correlated the induction of HSPs with a decrease in the expected injury from a subsequent stress. We have developed a model of cortical neuronal protection that allows direct comparison of a protected hemisphere with the contralateral, unprotected hemisphere. We used a distal middle cerebral artery occlusion in the rat as a priming insult to stimulate a stress response in the cerebral cortex with or without causing neuronal necrosis. Twenty-four hours later the animal was subjected to a ten minute period of bilateral common carotid artery occlusion with hypotension in a temperature and humidity controlled environment. This global ischemic insult has been characterized to result in symmetrical, selective cortical and subcortical neuronal necrosis in unprimed animals. In a preliminary set of three rats, we found a decrease in the number of necrotic neurons in the primed cortex compared to the unprimed side in two animals. In the third animal there was insufficient cortical injury on either side to detect protection. Subcortical structures were symmetrically affected in all three animals. This model suggests a correlation exists between the in-vivo induction of HSPs and resistance to ischemic cell death. The presence of a side-to-side comparison of the ipsilateral affected, and a unilateral control (adjacent cortical and subcortical regions), makes this an attractive model for the further characterization and enhancement of neuronal protection during stress.

524.6  
HOMEOGENEASE mRNA AND PROTEIN LEVELS INCREASE IN NEURONAL CELLS IN RESPONSE TO HYPOXIA AND HYPOXIA-REPERFUSION OXIDATION. C.M. Bills, B.J. Murphy and L. Tolst, Department of Neuroscience, SRI International, Menlo Park, CA 94025

The presence of increased amounts of oxidized proteins in aged animals, particularly in the brains of such animals, have led to the discovery that compounds which react and stabilize free radicals generated during such oxidations can reverse degenerated behavioral parameters in older gerbils to levels comparable to those of young gerbils. In aerobic animals, a number of natural antioxidants exist which include superoxide dismutase, catalase, and the blue pigment, bilirubin. We have found that during a hypoxic episode in immobilized neuronal cells, homogenase (HO), the enzyme responsible for the generation of bilirubin, and the mRNA which encodes it, increase several-fold. Since free radicals are most prevalent in the brain following an ischemia-reperfusion episode, we mimicked this situation by reoxygenating the cells following hypoxia and found that the increased levels of HO mRNA are maintained. Interestingly, the regions of the brain most vulnerable to hypoxia-reperfusion are areas in which HO mRNA is most abundant. We propose that the generation of the antioxidants biliverdin and bilirubin protects the brain from the assault of free radicals generated during ischemia-reperfusion, and the extent to which homogenase is induced contributes to the resiliency or vulnerability of the brain (particularly, the hippocampus) to ischemia.

524.7  

SGP-2 is emerging as an important marker for neurodegeneration. In this study, we examined SGP-2 RNA expression in rat hippocampus (HC), caudate nucleus (CN), and cortex (CX) 24 hours after transient global ischemia induced by four vessel occlusion (4VO). At 72 hours post-4VO, SGP-2 RNA is elevated in HC, CN, and CX (May et al. Mol. Brain Res., in press). However, at 24 hours post-4VO, SGP-2 RNA is increased only in CN and CX (1.5- and 7-fold, respectively); there is no change in SGP-2 RNA in HC. Induction of SGP-2 RNA at this early timepoint is limited to regions (CN and CX) that were most rapidly neurodegenerative post-4VO takes place; this induction has not yet occurred in HC, where neurodegeneration is delayed. To further study the relationship between SGP-2 and neurodegeneration, we characterized the effects of a selective inhibitor of LY231617, an antioxidant compound that is protective against ischemic damage in HC and CN, in preventing SGP-2 RNA expression following 4VO. LY231617 (50 mg/kg) or vehicle was administered orally before and after 30 minutes of 4VO, and the rats were sacrificed 72 hours later. Treatment with LY231617 prevented the induction of SGP-2 RNA in HC but not in CN, although 4VO-damage in both regions was markedly attenuated by the compound. These data suggest that ongoing neurodegeneration is not an absolute requirement for the induction of SGP-2 RNA in the brain.

524.8  
INDUCTION OF FOS AND JUN EXPRESSION FOLLOWING FOCAL CEREBRAL ISCHEMIA-REPERFUSION INJURY. G. Ah, T.N. Li, J. Xu, Y. He, C.Y. He*, Division of Restorative Neurology, Baylor College of Medicine, Houston, TX 77010.

Alterations of immediate early gene expression have been observed during central nervous system ischemia. In the present study, the expression of the proto-oncogenes, c-fos, c-jun, Jun B and Jun D were investigated in a rat focal cerebral ischemia-reperfusion model. Northern blot analysis indicated that the expression of c-fos and Jun B in control brains were very low and both were co-induced immediately following focal cerebral ischemia-reperfusion and peaked at 30 and 60 min after reperfusion respectively. c-jun was expressed constitutively in a significant level in all time intervals examined and was also enhanced in a similar pattern as c-fos and Jun B but with a much lower magnitude. In contrast, no change in jun D expression was observed. The increases of c-fos, Jun B and c-jun mRNAs were localized to the cortex and hippocampus, as shown by in situ hybridization. Northern blot analysis indicated that the increased mRNA levels of c-fos, Jun B and c-jun are due to the increases of transcription rate in these genes. Mobility shift assays were used to determine the DNA binding activity of transcriptional factor AP1 (Fos-Jun heterodimer). Nuclear extract from control cortex showed a basal binding activity to the AP1 DNA sequence; however, 90 min ischemia followed by 4 hr reperfusion resulted in a 4 to 6-fold increase of AP1 binding activity, and the enhanced DNA binding activity persisted for at least 24 hr. These results suggest that, as general transcriptional factors, the changed expression of fos and jun may play a central role in post-ischemic alteration of genetic process.

524.9  

The expression of c-fos and c-jun mRNAs was localized by in situ hybridization in the transperitoneal hypoxic (H/H) brain injury (Rice et al., 1981). Rat pups (PND7) were exposed to unilateral common carotid ligation, followed by 3 h of hypoxia (85%/2%/92%) in the left hemisphere with or without damage to the right hemisphere. Probes for c-fos and c-jun mRNAs. At 2 h post-hypoxia, c-fos and c-jun expression was observed in ipsilateral (I) striatum and hippocampus, as well as in both vulnerable and spared regions of the cortex. A columnar pattern of expression was observed in the ipsilateral MCA cortex, which is similar to the pattern of permanent morphological damage observed in this area in neonatal but not adult, models of unilateral H-I. Immunohistochemistry in these areas in these animals, expression in the contralateral hemisphere occurred in areas that represented a partial mirror image of those on the I side. The ipsilateral striatum, MCA cortex, and hippocampus exhibit selective vulnerability to damage in the neonatal model. Since fos and jun mRNA expression occurs in both vulnerable and spared regions, AP1 transcriptional activity could mediate region-specific responses to H-I in the immature brain. Sup. by Coleen Glbfn Fdn. and NS28363.

524.10  

Immediate early gene (IEG) products, such as FOS and JUN, may partially mediate the long-term transcriptional response of CNS cells to specific changes in their environment. To determine whether IEG products might play a role in the immature brain’s response to hypoxia-ischemia (H-I), rat pups (PND7) were subjected to unilateral common carotid ligation followed by 3 h of hypoxia (85%/2%/92%) at 37°C, which results in pathological changes only in the ipsilateral hemisphere (Rice et al., 1981). Following H-I, 3 animals were euthanized at each of 4 time points (1, 12, 18, and 24 h). RNA from 4 brain regions were analyzed by northern blot hybridization for their relative concentrations of 9 IEG mRNAs (c-fos, c-jun, b-actin, and TIS 1 (nur77), TIS7, TIS2 (zosteth), TIS50, TIS11, and TIS21). Induction of all 7 IEGs, except TIS7 and TIS10, was observed in ipsilateral forebrain, and less frequently in contralateral forebrain, at 1 and 2 h post-hypoxia. In some animals, low levels of expression were also detected at 18 and 24 h. In a minor exception, induction of all 7 IEGs was observed in a given RNA sample. In summary, expression of neonatal rats to H-I produces a dramatic induction of 7 IEG mRNAs in their brains. Several of these are also expressed in hypoxic/fetal (fos expression, before PND20 (Sakurai-Yamashita et al., 1991)). Thus, the extensive IEG response to hypoxia in this model represents one of the few reported thus far in which IEG-encoded proteins could mediate long-term cellular responses in neonatal brain. Sup. by Coleen Glbfn Fdn. and NS28363.
Neurotrophins (NTs) have been shown to promote the differentiation and survival of certain types of neurons and may play a role in plasticity and regeneration after CNS injury. In this study, the influence of focal cerebral ischemia on NT family mRNA expression was examined using Northern blot analysis and in situ hybridization. Transient focal cerebral ischemia for 30 minutes with reperfusion of 30 minutes or 4 hours induced a transient increase in NGF and BDNF expression in ischemic cortex. The peak was found two to four weeks after ischemic insult. The biphasic expression of NT mRNA was also observed in the contralateral cortex which sustained only very mild ischemia. Similar biphasic NT mRNA expression was also noted when ischemia lasted for 90 minutes leading to a permanent infarct. Interestingly, in hippocampus, a monophasic peak happened between four hours to 1 week after reperfusion, which corresponded to a period of depressed NGF and BDNF expression in the ischemic cerebral cortex. There was no obvious increase in NT-3 expression. We also failed to detect CNTF mRNA signals in brain with or without ischemia. Results from this study suggest time-dependent expression of selected NTS.

The derenation in the cortex following damage from ischemia should provide a stimulus for sprouting. Earlier work supported the existence of synaptogenesis after cortical infarction using antibodies to synaptophysin. The present study demonstrates the distribution of immunoreactivity against GAP 43, a growth cone associated protein, as an indicator of neurite growth following cortical infarction. Hypertensive rats were subjected to permanent transient occlusion of the right common carotid and right distal middle cerebral arteries. This procedure produces ischemia in the cortex with little damage to subcortical structures. After recovery times of three days to two months, the animals were perfused with paraformaldehyde and processed for conventional immunohistochemistry using monoclonal antibodies to GAP 43. These slices were evaluated on a Quantex image analyzer to determine the optical density of the reaction product. Preliminary optical density data is shown below (* P < .01 Student's t-test): Time Post-Op O.D. (iu) to Infarct O.D. contra to Infarct 3 days * 17.0±0.5 8.0±2.3 7 days * 47.0±6.1 30.1±6.6 14 days * 41.0±7 28.3±4.5 1 month 17.3±10.6 19.0±7.3 2 months 40.4±9.1 49.0±4.7 These results suggest that neurite sprouting occurs shortly after ischemia in the cortex, but is diminished by one month when synaptogenesis occurs. This supports the hypothesis of neurite growth and synaptogenesis in the cortex following ischemia. Supported by NS 11255, NS 01217, and Bristol Myers-Squibb.

We have shown that following hypoxic-ischemic (HI) injury to the rat brain, there is a marked induction of mRNA for IGF-1, IGF binding proteins B2-4 and TGFβ in the areas of neuronal loss within 5 days. Induction of NGF-B, BDNF or neurotrophin-3 was not detected. IGFBP-4 is known to be a non-competitive neurotrophic agent. We examined the possibility that IGF-1 may be neuroprotective. Rats were subject to a unilateral HI injury by ligating one carotid artery then subjecting the animals to 10 min of 5% inspired O2. This induces considerable neuronal loss and infarction in the middle cerebral artery territory of the ligateded hemispheres. Growth factors and vehicle were administered as bolus injections into the lateral ventricles.
IGF-1 given 2 hours after the insult reduced the infarct rate. In controls the infarct rate was 85%; this reduced to 36% with 5µg IGF-1 (n=14) and to 27% with 50 µg IGF-1 (p<0.05, n=15). Similarly within each neuronal region IGF-1 caused dosed dependent neuroprotection particularly in the striatum and cortex. IGF-1 treatment did not alter cortical temperature. Equimolar doses of insulin did not improve outcome. Indicating its leading to a permanent IGF-1 does not act via the insulin receptor or hypothemic mechanisms. This peptide may have therapeutic use in the rescue of the injured brain.

524.16 IDENTIFICATION OF CHANGES IN NUCLEOTIDE INTERACTING PROTEINS IN CONTROL VS ISCHEMIC RAT BRAINS Banamathi Ranganathan, Bond Walzer* and James Clemens, R. Lilly & Co., Indianapolis and College of Pharmacy, University of Kentucky, Lexington, KY, 40536.
The effects of ischemia on the phosphorylation and nucleotide binding properties of rat brain proteins were examined. Tissue homogenates were from the corpus striatum, dorsal hippocampus and pedunculopontine tegmental regions. Samples from both control rats and rats subjected to 30 minutes of 4°C followed by no reperfusion, 1 or 24hrs reperfusion were phosphorylated or photoabeled. Either or γ32P labeled 8H4TP or 8H4GTP were used. Major changes in phosphorylation and nucleotide binding of specific proteins in ischemic rat brains were observed. Most changes were observed in the striatum, a few in the hippocampus and almost none in the cortex. This agrees with the fact that after the ischemic insult, striatum degenerates in 24hrs, hippocampus dies after 3 days and cortex shows minimal degenerative changes. Changes in phosphorylated proteins in ischemic brain proteins of 55-57 (Ca2+ kinase), 42-43 (cAMP kinase), 31 (unknown, but also a GTP binding protein) was observed. The effect of IGF-1 was also studied. Supported by R. Lilly & Co. & NIH grant GM-35766-07.
GLOBAL CEREBRAL ISCHEMIA EFFECTS ON HIGH MOLECULAR WEIGHT PEPTIDES AND CA1 PYRAMIDAL NEURONS IN THE RAT HIPPOCAMPUS.


Department of Neurosurgery and Anest, Univ. of Koch, and Viscon Tech, Rochester, VA, New Orleans, LA.

The four-vessel occlusion (4-VO) rat model of global cerebral ischemia has been used widely to study selective hippocampal cell vulnerability, gliosis, and viability or plasticity of surviving neurons in the molecular events of selective CAI vulnerability and the plasticity of surviving neurons in response to injury. The specific aim of this study was to correlate the post-ischemic progression of CAI cell death and the molecular events which may play a critical role in neuronal vulnerability and plasticity in response to injury.

Reverse phase HPLC was used to profile hippocampal peptide changes following 4-VO. Rats were sacrificed 1, 3, or 7 days after 4-VO or sham surgery. Supernatants of denatured hippocampal homogenates were passed through G50 Sephadex columns and the high molecular weight fraction separated by HPLC. Peaks which exhibited a 25% or greater change after 4-VO were isolated for identification.

Trypsinized peptides were sequenced and identified by homology match to GenBank. In two rats from each group, CA1 pyramidal neurons were counted in three anatomically-defined regions of CA1, in two sections of the dorsal hippocampus.

The number of CA1 pyramidal neurons was not decreased significantly on day 1 after 4-VO, but was on days 3 and 7. Peptides that exhibited a relative change of 25% or greater were: zinc binding protein (25, 23, -25%, days 1, 3, 7); myelin basic protein (-7, -38, -28%), serum albumin (31, 58, 76%), calmodulin (8, 33, -19%), alpha hemoglobin (28, 50, 16), and beta hemoglobin (62, -11, 21%). The correlation of post-ischemic CAI cell death with specific peptide changes provided evidence on the critical role of specific molecular events in neuronal vulnerability and plasticity. Supported by NIH AG 00170 and LEAD Award AG 0091 at FDC.

Following cardiac arrest and resuscitation an in situ reaction of thiobarbituric acid (TBA) and fluorescent microscopy were used to localize post-ischemic peroxidative damage in the rat forebrain. After reperfusion (90 or 240 min) the brains were fixed transectionally with 50% aldehyde-free methanol, 10% acetic acid, 2nM EDTA and 0.375% TBA. Formation of TBA-malon-dialdehyde adduct (TBA-MDA adduct = 515 nm) was assayed in serial 40 μm frontal sections. Brains of normal and ischemic-only groups provided the controls. Intense granular fluorescent was found within the cell body of neurons in many of the selectively vulnerable zones (SVZ) after either reperfusion time. Fluorescent granules were detected primarily in the perinuclear region consistent with the COX apparatus, and were absent in glial cells. The dorsolateral sector of the cerebral cortex at rostral levels contained the largest numbers of fluorescent neurons (FNS) which were distributed heterogeneously throughout the layers. Numerous FNS were also found in the pyramidal layer of CA1 (CA, CA2, and hilar region) but only a few FNS were detected in the dorsolateral striatum and septal nuclei. The present data indicate that neurons in the SVZ are targets of peroxidation during reperfusion. It further suggests that, within a zone, a time dependent vulnerability during reperfusion may exist among neurons of different morphological and chemical phenotypes. (Supported by NIH grants NS24819 and GM8107).


Damage as result of ischemia subsequent or ischemia affects the substantia nigra, the hippocampus, striatum and cortex more than other brain regions. The cause of this cell death is unknown. In ischemic models of radial generation plays an important role. And although defense mechanisms are present in these brain areas, these may be insufficient to counteract with the large radical general, therefore protective radical generating circumstances as an MPTP injection. In in vitro studies of capacity of homogenized samples of different brain regions are controversial. In vivo studies, describing expression of these enzymes in the rat brain are also not available. We tried to localize glutathione reductase which reduces oxidized glutathione (GSSG) to glutathione (GSH). The hypoxia sensitive CA1 region in the hippocampus shows a high glutathione reductase appearance compared to the CA2/CA3. Furthermore the cells in the zona compacta of the substantia nigra, probably dopaminergic, were labeled. In this region the reticulata a strong extracellular labeling was found with only few positive neurons. In the dorsolateral striatum many neurons expressed this enzyme. All CA1, CA2, lateral, and globus pallidus showed faint cellular staining. This phenomenon might explain some features found after ischemia.

ATRIAL Natriuretic Peptide Blocks Proteolysis of the Blood-brain Barrier by Bacterial Collagenase in Rat. G. A. Rosenberg* and E. Estrada. Neurology Service, Veterans Affairs Medical Center, Department of Neurology and Physiology, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

Atrial natriuretic peptide (ANP) is important in regulating brain fluid balance and metabolism. We have shown in collagenase-induced hemorrhages that brain edema is present by 4 hours and worsens over 24 to 48 hours before recovery begins; the BBB is opened by 30 min. Therefore, we studied the effect of intravenous or intraperitoneal injection of ANP on edema formation and on the BBB. Adult rats (n = 6) had lesions produced in the caudate/putamen by the stereotactic injection of 0.4U of bacterial collagenase in edema formation and on the BBB. Adult rats (n = 6) had lesions produced in the caudate/putamen by the stereotactic injection of 0.4U of bacterial collagenase. Lesions in rats with lesions from 12 to 32 days with rats without ANP (n = 6) had ANP (5 μg/kg/hr) infused intravenously for 40 minutes 1 hour prior to the lesion; the permeability of the BBB was studied by the intravenous infusion of 125-I-collagenase given 10 min before the end of the experiment. Five control rats were similarly studied but without ANP. ANP significantly reduced the percent water at the injection site from 2.39 ± 0.24 to 0.55 ± 0.35 and the sodium from 402 ± 17 to 399 ± 15 nmol/mg dry wt (p < 0.002). The percent uptake of 125-I-collagenase from blood into the brain was reduced from 17.3 ± 2.4 to 12 ± 2.4 in rats with ANP (p < 0.0004). We conclude that intravenous ANP is effective in reducing brain edema in a hemorrhagic lesion by protecting the capillary from proteolytic damage by bacterial collagenase. ANP may be useful in treatment of brain edema.

FASTIGIAL STIMULATION ENHANCES EEG RECOVERY AND REDUCES TISSUE DAMAGE AFTER FOCAL ISCHEMIA. E. Zhou and C. Jadaclo. Dept. of Neurology, Univ. of Minnesota, Minneapolis, MN 55455.

Stimulation of the cervical and caudal fastigial nucleus (FN) increases extracellular cerebral flow (CBF) but not metabolism and reduces the brain damage resulting from focal ischemia (CBF&h(M.8.0, 1991). The FN may exert this effect by enhancing CBF to the ischemic without increasing local metabolic demands. Furthermore, to test the hypothesis we studied whether the protective effect is restricted to the neocortex, a region in which the CBF increases, but not to the thalamus, where there is no change in CBF. In 38 rats, the FN was stimulated during ischemia, and the time to reestablishment of the dorsal medullary reticular formation (DMRF), a tumor rat increases CBF and metabolism, also reduces the brain damage resulting from ischemia. In halothane anesthetized (1.3%) the FN was stimulated extracellularly with 20 mHz or 10 mHz and reduced either area in the neocortex (30-100x4mm) and striatum (51x8) (Mean±SD). Distal CMA occlusion (m) produced infarcts restricted to the neocortex (2312±8). FN stimulation substantially reduced stroke size (p<0.001). The reduction was greater after distal (69±5%) than proximal (48±5%) MCA occlusions (p<0.001) and occurred in neocortex (43±7%) (p<0.001) and in the striatum (35±7%) (p<0.001). FN stimulation reduced EEG amplitude in the ischemic cortex by 73±1%. One hour after the EEG recovery were 13% in unstimulated and by 48% with FN stimulation (p<0.001). FN stimulation (m) did not affect stroke size or EEG recovery (p=0.05). The FN stimulation, but not DMT, attenuates the functional impairment and tissue damage resulting from cerebral ischemia. The finding that the protection is limited to the neocortex and that the amount of tissue salvaged is greater after distal MCA occlusion suggests that the FN may protect ischemic tissue from infarction by enhancing collateral flow without increasing local metabolic demands. Activation of selected neural networks protects the brain from the consequences of focal cerebral ischemia. (Supported by the American Heart Association of Minnesota).

LEUKOCYTE BEHAVIOR DURING ACUTE REPERSUSION AFTER GLOBAL CEREBRAL ISCHEMIA IN THE RAT BRAIN CORTEX MICRO-CIRCULATION. G. Sten, A. Villberg, U. Linna. Dept. of Neurology, University of Munich, 8000 Munich 70, F.R.G.

We tested the hypothesis that leukocytes (WBCs) are activated within the brain microcirculation by global ischemia, and that obstruction by WBC is responsible for postischemic hyperperfusion. Wistar rats were anesthetized with Inactin, tracheotomized, ventilated and a closed cranial window was performed to expose the dorsal spinal cord. Using a stereotactic frame with intraperistopic magnification, a needle-tip fiberoptic probe was placed over the dorsal horn of the cord. Ventilation was adjusted to maintain the PaCO2 at 3.3 mmHg at one of three predetermined levels (30, 40, 50). SCBF was averaged over two minutes of continuous monitoring. Segmental SCBF at PaCO2 levels of 30, 40, and 40 mmHg were 58.8, 96.2, and 105.4 cc/mg/min, respectively (p < 0.0001). Linear regression revealed that the slope was 2.74 when the PaCO2 changed from 30 mmHg to 50 PaCO2 (p < 0.0001). Laser Doppler flowmetry is a useful tool in measuring relative changes as well as continuous online sampling of regional SCBF in the rat.

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The endothelial adhesion of monocytes and expression of the intercellular adhesion molecule-1 (ICAM-1) in the carotid arteries of rats with and without the stroke-risk factor hypertension were demonstrated using the immunofluorescence technique. Perfusion-fixed frozen sections (16 μ) of carotid arteries of spontaneously hypertensive rats (SHR), stroke-prone SHR (SHR-SP) and normotensive Wistar-Kyoto (WKY) rats were exposed to specific monoclonal antibody against rat monocyte/macrophages (ED-1) or rat ICAM-1. All slides were double stained with anti-Factor VIII antibodies for the identification of endothelial cells which showed no ED-1 staining. In SHR and SHR-SP, single monocytes or clusters of monocytes/macrophages were found adherent to the endothelium. In SHR-SP, ED-1 positive cells were present also in a subendothelial location. The number of endothelium-attached monocyte/macrophages per millimeter of internal elastic lamina was significantly greater in SHR-SP than in SHR (5.1 ± 0.7 and 3.2 ± 0.4, p<0.05, n=4). No ED-1 staining was found around the endothelium in WKY rats. Patchy expression of ICAM-1 on endothelial cells was found in carotid arteries of SHR and SHR-SP, but not in WKY rats. The results suggest that the increased accumulation of monocyte/macrophages and activation of endothelial cells are associated with the stroke-risk factor hypertension increasing the tendency for the endothelium to convert from an anticoagulant to a procoagulant surface.

526.7 RESPONSE OF CEREBRAL CAPILLARY ENDOTHELIAL CELLS TO HYPOXIA AND RADIATION J.T. Gobel, T.Y. Chan, J.R. Pike, P.H. Chase; CNS Injury & Brain Edema Res Center, Dept. Neurol, & Brain Tumor Res Center, Dept Neuropsych, Univ. of Calif., San Francisco, CA 94143.

Nitric oxide synthesis inhibitors, such as nitro-L-arginine, and polyamine inhibitors, such as α,α-difluoromethylornithine (DFMO), can inhibit ischemic and/or radiation-induced brain injury. The cellular basis of these effects may be related to inhibition of NMDA receptor activation. We have examined the effect of both radiation and hypoxia on cerebral capillary endothelial cells and modification of that effect by nitro-L-arginine (0.5 mM) and DFMO (1 mM). Cells were isolated from 14 day old Sprague-Dawley rats. At 4.7 days after plating, cells were treated with 24, 48, or 62 hrs of complete hypoxia or a 16, 32, or 64 Gy radiation dose. There was an initial increase in protein content detected by an increase in lactate dehydrogenase (LDH) within the media, at 48 hrs of hypoxia, and the release of lactate dehydrogenase (LDH) intensified with increasing time of hypoxia or increasing radiation dose. The hypoxia-induced LDH release correlated with an increase in the ratio of dead to viable cells (ethidium bromide and fluorescein isothiocyanate staining). While DFMO did not reduce the release of LDH due to radiation, it did appear to reduce radiation-induced shedding of endothelial cells from the substrate. Neither DFMO nor nitro-L-arginine seemed to alter hypoxic injury under our experimental conditions. DFMO inhibited a hypoxia-induced increase in total LDH measured within cells attached to the substrate, suggesting that hypoxia either induces an increase in intracellular LDH or stimulates cellular proliferation. These results suggest that activation of NMDA receptors are not involved in hypoxia-induced injury to cultured cerebral endothelial cells. In addition, the modification of radiation-injury by DFMO may be related to a reduced rate of release of damaged endothelial cells and thus maintenance of the endothelial cell layer. Supported by NIH Grants AG 08938, NS-14543, NS-25372, and National Brain Tumor Foundation.

526.8 ATP-SENSITIVE POTASSIUM CHANNELS IN RAT BRAIN MICROVASCULAR ENDOTHELIAL CELLS Damir Janigro*, Ellen L. Gordon & H.H. Richard Winn Dept. of Neurological Surgery, University of Washington, Seattle, WA 98194

The endothelium plays an important role in the modulation of vascular tone and blood cell activation. Extensive work has demonstrated that the release of EDRF from the endothelium is evoked by a number of physical and chemical stimuli requiring Ca2+. Since endothelial cells do not express voltage-dependent calcium channels, calcium influxes following receptor activation may be facilitated by cell hyperpolarizations mediated by the activation of potassium conductances. We have investigated the electrophysiological properties of an ATP-sensitive K+ conductance in whole cell and membrane patches from rat aorta and brain microvascular endothelial cells. Whole cell as well as patch currents were increased by either intracellular dialysis of ATP or by application of glucose-free/NaCN (2 mM) solutions. Both currents were reversibly blocked by glibenclamide (1-100 μM). The K_{ATP} channel opener pinacidil (30 μM) caused activation of an outward current in the presence of physiological [ATP]. In inside-out patches, ATP (10 μM-1 mM) invariably caused a dramatic decrease in channel activity. We conclude that both rat aorta and brain microvascular endothelial cells express K_{ATP} channels. K_{ATP} may play a role in the regulation of endothelial cell resting potential during impaired energy supply and therefore modulate EDPR release.

526.9 EFFECTS OF THE CYTOTOXIC AGENT TIRILAZAD MESYLATE (U-74006F) ON THE TIME COURSE OF NEUTROPHIL INFILTRATION IN CEREBRAL ISCHEMIA J.A. Oosterveld* and L.K. Williams, CNS Diseases Res., The Upjohn Co., Kalamazoo, MI.

Using the gerbil unilateral carotid occlusion (UCO) model of transient cerebral ischemia, initial experiments identified a substantial accumulation of cytochrome oxidase (CO) - positive neutrophils (NL) in the ischemic hemisphere. After 4 hrs of reperfusion. The NL accumulation correlated with a severe neuronal death in the CA1 region of the hippocampus (H). Treatment with tirilazad at 10 mg/kg i.p. both immediately before and after the UCO resulted in a significant 64% reduction in NL accumulation, and a significant 25% reduction in the incidence of neuronal death. In the present experiments, animals were collected after 0 to 24 hrs of reperfusion, and frozen coronal sections were stained with CO and cresyl violet histochemistry. The incidence of neuronal death was scored semiquantitatively with a Viability Index (VI) of 0 (no damage) to 4 (complete neuronal loss). After 3 hrs UCO and 2 hrs reperfusion, beginning neuronal death was apparent (VI = 0.36 ± 0.10, n=11), but NLs were not observed. After 4 hrs reperfusion, neuronal death was the same (VI = 0.28 ± 0.12, n=14), and NLs were not observed. After 6 hrs of reperfusion, neuronal death was the same (VI = 0.78 ± 0.30, n=9). After 6 hrs of reperfusion, tirilazad treatment resulted in an 80% reduction in NL accumulation, and an 85% improvement in the VI (n=9). Thus, tirilazad may act directly on the endothelial cell layer to limit pericellular membrane damage and to limit chemotactic signaling, and may have a direct action on NLs to limit their activation, adhesion, diapedesis, or migration, thus limiting consequent NL-mediated oxidative neuronal destruction.

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SOTY 1
EARLY ISCHEMIC INJURY IN THE BASAL GANGLIA: AN IMAGING AND NEUROPATHOLOGICAL STUDY.

Early magnetic resonance imaging (MRI) and neuropathological examination in a model of regional ischemia in baboons. Models included complete ischemia (CI) and incomplete ischemia with reperfusion (IRI). The earliest changes in basal ganglia (BG) appeared in IIR as detected by MRI (three hours postischemia). Immunocytochemical studies included the analysis of neurofilaments, microtubule-associated protein-2 (MAP2), and calcium-binding protein (calbindin parvalbumin). The external portion of the striatum appeared to be the initial focus of necrosis. Loss of patch and matrix organization of striatum was one of the earliest changes, as demonstrated by a disappearance of compartmental distribution of MAP2. The magnitude of ischemic injury in IIR appeared to be more severe than CI. These findings suggest that disruption of neurotroskeletal organization is an early change during ischemic injury and that reperfusion enhances ischemic damage.

SOTY 3
EARLY DETECTION OF EMBOLIC STROKE BY CONTRAST ENHANCED MAGNETIC RESONANCE IMAGING. K.H. Taber*, S.R. Northrup, J. Kirkpatrick and I.A. Bayman. Department of Radiology and Biotechnology, Baylor College of Medicine, Houston, TX 77030.

Sequential magnetic resonance (MR) images were acquired at 2.35 Tesla in rabbits 30 minutes to 6 hours after induction of embolic stroke. The normal evolution on T2 weighted images was a gradual appearance of bright signal, with the area(s) of stroke moderately well delineated by 2-4 hours after onset. Contrast-enhancement with GD-DTPA resulted in development of extremely bright signal on both T1 and T2 weighted images by 30-60 minutes after administration. This delayed appearance of the contrast agent is gradually pooling in the stroke tissue. Comparison of the bright areas on the MR images with the areas of stroke as determined by TTC staining indicated that the stroke was accurately portrayed. Thus, contrast administration can be used in this model of stroke to allow earlier identification of the affected area than is possible on non-enhanced MR images.

SOTY 4
DIFFUSION MR IMAGING: CORRELATION WITH HISTOPATHOLOGICAL FINDINGS IN EVOLVING STROKE. C Pierpaoli, A. Biglino, C Ferresou, F. Flaviano, R. Mbich* and J.B. Ager, G.B. Chen. Neuroimaging Branch, NINDS, NIH Bethesda MD 20892

Conventional T2 weighted magnetic resonance imaging (T2WI) is able to detect the vasoergic edema developing during cerebral ischemia but is inadequate for the evaluation of the size, severity and location of the lesion within the edematous area. In this study we investigated the ability of diffusion-weighted MRI (DWI), a technique sensitive to the Brownian motion of water molecules, to offer more specific information about the histopathological changes encountered in the evolving and complete stroke. Focal cortical infarction was induced in rats by two minute occlusion of the middle cerebral artery. DWI appeared to the early occurrence of extensive bright signals on T2WI. At 15-20 minutes after reperfusion the signal intensity decreased significantly from normal. DWI was able to identify changes in the injured area as soon as 20 minutes after the induction of the ischemia. Thus, DWI may provide a clearer discrimination among the necrotic core, a surrounding rim in which the tissue architecture was still preserved and the peripheral edematous gray matter was observed at 24 hours. Our data suggest that DWI may be suitable for improving diagnostic capabilities as well as for testing 'in vivo' the effectiveness of stroke therapy.

SYMPOSIA
THURSDAY AM

SYMPOSIUM. MOLECULAR BIOLOGY OF OLFACTION. G.M. Shepherd, Yale (Chairman); R.R. Reed, Johns Hopkins; L. Buch, Harvard; R. And, Columbia (Chairman).

The molecular mechanism of odor discrimination has long been a central unsolved problem in sensory neurobiology. The last decade has seen enormous progress, from a series of studies of olfactory sensory receptors, and the molecular understanding. These developments have culminated recently in the molecular cloning of candidate olfactory receptor genes. The purpose of this symposium is to convey the excitement generated by the recent advances, and to summarize current understanding of the molecular basis of olfaction, and delineate the areas within this new knowledge impacts on the neurosciences.

G.M. Shepherd will provide an overview, correlating the molecular physiology and pharmacology of olfactory transduction with the molecular components of the transduction pathways, and delimiting the implications of the new studies for neural mechanisms of odor processing. R.R. Reed will summarize his studies in cloning and sequencing the G protein, adenylate cyclase, and membrane conductance channel in the sensory transduction pathway in the olfactory sensory neuron. He will describe his current work in identifying new members of the receptor gene family. L. Buch will describe the innovative strategy and methodology used in cloning and sequencing the large gene family for the candidate receptor proteins belonging to the 7 transmembrane domain receptor superfamily. She will discuss the complexities of the olfactory receptor repertoire in mammals. R. Axel will summarize the extension of his collaboration with Buck to a comprehensive characterization of the molecular and cellular organization of a fully tractable olfactory system in fish. He will discuss the implications of this model for the organization of the peripheral sensory pathway. B. Lanzet will discuss mechanisms for olfactory signal modulation: desensitization, possibly by receptor phosphorylation, and termination, by binding proteins, cytochrome F-450 and UDF glucuronosyl transferase. He will discuss the expression patterns of human olfactory receptors, with implications for diversity and polymorphisms in human odor recognition.


The goal of this symposium is to present and discuss several recent and exciting developments in our understanding of the mechanisms involved in neurosecretion. For decades, the neurophyophyseal system has served as a model system for elucidating cellular mechanisms of neurosecretion which have proved, in general, to be applicable to other neuronal systems. Eva-Maria Dorsa will present evidence for transport and endogenous vasopressin (VP) and oxytocin (OT) mRNA expression is the hypophalamic to the posterior pituitary and discuss the potential for functional significance of these mRNA in the norepinephrine. Floyd Bloom will discuss the findings that hypophalamic microinjection of VP mRNA leads to selective uptake, retrograde transport and expression of VP exclusively in the magnocellular neurons of the Brezhalloret system leading to a posterior reversal of diabetes insipidus for up to 5 days. Fred van Leeuwen will present evidence for VP gene repair in Brezhalloret rats, and discuss the expression of VP mRNA. Celia Sladek will discuss in vitro approaches to studying the regulation of gene expression in the neurohypophyseal system including the role of second messenger systems, and Seddon Dorsa will present new information on the regulation of VP gene expression by hormone activated transcription factors. These new findings in the neurohypophyseal system promise to alter our view of the regulation of a posterior secretion by neurons in general.

A dual detector probe (Bae, A.N., J. Nucl Med 27:184-191, 1986) permits positron emission studies of the brain at much reduced radiation dose and cost as compared to conventional PET. Coincidence counts obtained from the device can be used either directly or in an MRI-registered computer model if measurement of activity within specific regions of the brain is desired. Studies of the correlation between of probe measurements and PET region-of-interest (ROI) data in humans have been carried out. In these studies, subjects were scanned with the probe immediately following a PET scan. Multiple studies were performed on each subject in either a haloperidol dose response or withdrawal paradigm. Haloperidol occupancy of dopamine D-2 receptors in the striatum was measured by its blockade of N-[3H]-methylnoradrenaline binding. The ratio of counts from a position directed at the striatum versus a position directed at the cerebellum was used for the probe measurement of receptor occupancy. The results of PET using the dual detector probe system can provide estimates of dopamine receptor occupancy that are useful for drug kinetic studies and reflect ratios obtained by positron emission tomography.


Advances in ultra fast image acquisition and in the application of contrast susceptibility effects have made it possible to perform dynamic physiological scanning with magnetic resonance imaging (MRI). We studied fourteen subjects during visual activation and during a dark control state. Scans were obtained every 2.05 seconds for two minutes using an unmixed GE SIGNA 1.5T scanner and a Turbo GRASS pulse sequence. Signal potentials from a surface coil (N=7) and a standard quadrature head coil (N=7) was used. A bolus of Gd DTPA was injected during each condition. Cines produced from the series of rapid scans clearly depict an arterial, parenchymal, and venous phases. A pixel by pixel analysis of serial concentration maps derived from the rapid scans revealed expected cerebral blood volume (CBV) differences between gray and white matter as well as significantly increased CBV in calcarine cortex during visual activation versus control state (average increase of 15.59%, t=2.674, p<0.05 for the surface coil and 20.29%, t=3.495, p<0.05 for head coil). These results confirm earlier findings and demonstrate that dynamic CBV can be assessed with MRI during physiological activation.


RT at NIH NHLBI

High-speed non-invasive MRI methods sensitive to changes in cerebral blood flow (CBF) and oxygenation have been used to generate real-time functional MRI maps of human visual and motor cortex activation. Two different techniques, which do not require the use of contrast agents, were used to study perfusion changes associated with neuronal activity. The first approach is sensitive to changes in blood flow and oxygenation. As regional increases in neuronal activity, the number of magnetic spins flowing out of an activated volume leads to a shortening of tissue T1 and, thus, higher signal in T1 weighted images. In the second approach, signal intensity is inversely proportional to the concentration of deoxyhemoglobin. As CBF exceeds local oxygen consumption, this leads to an increase in venous blood oxygenation and thus higher signal in T2* weighted images. Over 15 subjects underwent dynamic MRI imaging using an echo planar imaging (EPI) technique. Changes in blood oxygenation were detected using a gradient echo imaging sequence sensitive to variations in T2*. Signal intensity was measured in a pre-stimulus distribution phase and a post-stimulus phase. Typically, a series of 60-160 images were acquired in 5 minutes. The images show signal intensity changes as a function of time for a region of interest within primary visual cortex, during darkness and during 8 Hz visual stimulation. Our non-contrast agent dependent, non-invasive MRI methods can be repeated safely in human subjects, thereby expanding the temporal-resolution window of in vivo brain investigation.

532.4  CEREBRAL BLOOD OXYGENATION AND BLOOD FLOW IN HUMAN SUBJECTS: MRI EVIDENCE FOR DECORRELLING DURING FOCAL BRAIN ACTIVITY. C. Stern*, K.K. Kwong, J.K. Rakowski, J.W. Belliveau, T.J. Brady, B. Rosen Harvard Medical School and MGH-NMR Center, Charlots, MA 02129

High speed echo planar imaging techniques were used to track MR signal changes which reflect cerebral blood flow (CBF) and cerebral blood oxygenation levels. Gradient echo (GE) imaging sequences were used to track changes in blood oxygenation, while changes reflecting CBF were evaluated using an inversion recovery (IR) technique for both surfaces of the cortex. Regional activity was altered in primary visual cortex using 8 Hz pattern-flush visual stimulation. Activity within primary motor and somatosensory cortex was induced by active finger movements and passive tactile stimulation. In eight normal volunteers, the duration of the visual stimulus was alternated repeatedly between 15, 30, 60, and 90 seconds to examine the effect of prolonged neuronal activation on the recovery time course of the MR measurements. During the stimulation period, MR signal intensity rose significantly above baseline on both the CBF and oxygenation sensitive sequences. Following the cessation of stimulation, the GE signal fell below the pre-stimulus baseline (see Figure); whereas, the flow sensitive IR signal did not drop below baseline. The recovery time of the GE signal undershoot ranges between 30 and 50, and does not appear to be sensitive to variations in stimulus duration. The increased GE signal intensity during activation is strong evidence for increased postcapillary blood oxygenation, as the GE signal intensity is sensitive to changes in paramagnetic deoxyhemoglobin. In agreement with previous PET results, the results suggest that during normal neuronal activity, blood flow and oxygenation levels become uncoupled, with CBF far exceeding increases in O2 utilization. The relatively long recovery time course of the undershoot may reflect increased O2 extraction as a result of activity induced changes in pH.

532.5  QUANTITATIVE REGISTRATION OF FILM AUTORADIOGRAPHY AND ITS APPLICATION TO 3D ANALYSIS OF CEREBRAL BLOOD FLOW. W. Zhang*, J.Y. Chong, D.W. Smith and M.D. Ginsberg. CVDRC D4-5, Neurology, Univ. of Miami Sch. of Med., P.O. Box 019660, Miami, FL 33101

Rat models of MCA occlusion are highly useful approximations of ischemia hemispheric infarction in humans. Three-dimensional (3D) representation and calculation of ischemia infarction has long been attracting people's interest. Quantitative registration of serial autoradiographic images can provide detailed 3D information of the entire neural system. We analyzed and computed several existing registration algorithms and applied them to 3D data set of rat brains with MCA occlusion. The principal axes scheme characterized by its efficiency is based on the invariant moments which indicate the centers of mass and the orientations of serial sections. With the cross-correlation method, one image is held fixed, while the second is repositioned by translation to overlay the first in every possible position. The point of maximum cross-correlation provides the necessary information for correction of translational misregister. Since the algorithm repeatedly uses the pixel gray values, it does not require well-defined section shape and is less sensitive to noise. A novel image registration scheme using disparity analysis developed at this center is based on a linear affine model with four-point-to-point distance mapping. This method estimates scaling, translation and rotation parameters simultaneously with mapping accuracy up to 100 microns. Quantitative comparisons were carried out by the image registration using these algorithms. We also demonstrate the 3D shape of ischemia infarction at MCA occlusion studies.


We report an initial series of human studies using a novel SPECT technique to assess brain activation during regional cerebral blood flow (rCBF) due to cognitive activation. Two 123I-MP and Tc99mHMPAO are administered to the same individual during different tasks. Images of the two tracers can subsequently be separated using a high-energy resolution sampling circle, but the photospectra of the two radionuclides differ (140 KeV for Tc9m versus 159 KeV for 123I). A number of initial control studies were performed with the two tracers administered simultaneously, or at different times, respectively. The performance of the same cognitive task. These studies indicated that the two tracers distribute similarly in the brain under similar physiological conditions. The variability in distribution in vivo was similar to that found in phantom studies. In two subjects we then administered HMPAO during an eyes-closed baseline task and MP in during visual checkerboard stimulation. Ratio images comparing baseline to activation condition showed a localized increase in rCBF in the occipital lobe. In two additional subjects we administered HMPAO during an easy baseline task and MP during a difficult task involving dichotic listening with directed attention instructions. The ratio images showed hemispheric asymmetry, with increased blood flow to the left temporal lobes while subjects listened for the semantic target. The dual-isotope technique permits analysis of brain activation during different tasks with absolute anatomical registration of the resulting images. This strategy applies promising for study of localization of cognitive processing in normal subjects and in groups suffering from psychopathological conditions such as schizophrenia.

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532.7
DECREASED GLUCOSE METABOLISM IN NORMAL AGING. N.J. Jastreboff, E.C. Richardson*, T.E. Neudahl, N. Kuske, B.B. Reed. Center for Functional Imaging, Lawrence Berkeley Laboratory, University of California, Berkeley, KA 94720, and University of California, Davis, 95616.

Decreases in both regional cerebral blood flow (rCBF) and regional cerebral metabolic rates for glucose (rCMRg) reportedly occur in normal aging, although this remains a controversial area. Here we present the results of a PET study of rCMRg in 6 young (mean = 27.5 ± 4.6) and 8 old (mean = 66.5 ± 5.6) normal subjects using a high resolution (2.6 mm in-plane) PET tomograph and 18F-fluorodeoxyglucose (FDG). We scanned these contiguous tomographic levels through the temporal lobes. Previously determined rate constants and an operational equation were used to determine rCMRg. The rCMRg values for temporal lobe regions were averaged over the three tomographic levels.

The results of a repeated measures analysis of variance revealed that, while the main effect of age was not significant (p = 0.075) there was a significant (p = 0.001) group x region interaction indicating that the pattern of metabolism differed for the two groups. A series of Bonferroni corrected t-tests revealed that the older controls had significantly lower rCMRg only in the right and left anterior temporal cortex. Decreases in rCBF have also been reported for the temporal cortex in aged controls, and more severe decreases in both rCBF and rCMRg occur in age-related diseases such as Alzheimer's disease. Thus, the current findings may reflect either a region-specific rCMRg decrease that occurs with normal aging, or early indications of cognitive dysfunction that is associated with age-related disorders.

532.8
REGIONAL CEREBRAL BLOOD FLOW (rCBF) AND GLUCOSE METABOLISM (rCMRg) AT REST AND DURING ACTIVATION IN HEALTHY HUMAN AGING ASSESSED BY POSITRON EMISSION TOMOGRAPHY (PET). E. Patella*, B. Horwitz, C.L. Gradis, J. Märtens, A. González-Avilés, E. De Micheli, St. Rapopor, M.B. Schapiro. Lab. of Neurosciences, NIND. Inst. on Aging, Bethesda MD 20892.

To understand the effects of age on rCBF and rCMRg, we studied two groups of healthy men (6 young: mean age 24 ± 2 yr, range 22-26; 5 older: 65 ± 5 yr, range 60-74) using PET with [15-OH]-water and [18F]FDG. In the same scanning session, subjects were studied under two experimental conditions (EC): at rest (eyes closed, minimal room noise) and during sensory activation (SA) (watching a documentary film), using a Scanditronix PC-2048-150 (74X54 mm) PET scanner, and a double FDG injection procedure (Brooks et al., J Nucl Med 28; 53, 1987; Chang et al., J Nucl Med 28; 852, 1987), which allows two sequential FDG injections. rCBF and rCMRg values were measured in absolute units of ml/100g/min and mg/100g/min, respectively. To reduce inter-subject variability, data were "normalized" to mean rCBF (rCBF) and rCMRg (rCMRg) values, respectively. Effects of age, EC, and age x EC were analyzed by ANOVA. Significant increases of absolute and normalized rCBF and rCMRg values were found during SA bilaterally in the occipital regions in both age groups. Significant age and age x EC effects were shown by frontal regions: at rest, rCBF and rCMRg were higher in the young than in the old; SA increased the young and decreases in the old, so that differences between the two groups increased during SA. Coupling between rCBF and rCMRg was present in both age groups and was stronger during SA. Although the mean of the differential effects induced by SA on rCBF and rCMRg in the frontal areas in young and old subjects is not clear and needs to be further investigated in a larger sample, these results suggest that rCBF and rCMRg undergo more age-related changes in frontal than in other brain regions, and support brain activation as an ideal condition to enhance differences in brain metabolism between young and old subjects.

533.1
DIFFERENT VULNERABILITY OF HIPPOCAMPAL GABA, PARVALBUIN AND CALCIUM IMMUNOREACTIVE FIBERS TO METABOLIC DECREASES WITH AGING. N.S. Dell'Amico, M.S. Collin, M. Malinari, M. Magrini, G. Collu, M.S. Rodríguez-Anguita. Experimental Neurology Lab., Inst. of Neurology, Catholic University of Rome, Rome (Italy).

In adulthood, axonal lesions of the hippocampus primarily affect GABAergic neurons while calcium binding proteins (CPP) containing neurons are relatively spared. It has been recently demonstrated that neuronal axons are more vulnerable than dendrites to metabolic decreases in the aged hippocampus (Dell'Amico et al., Behav. Brain. Res., 1993; 45, 125-134). The neuronal death caused by stress is still largely unknown. The present study was aimed at investigating the effects of metabolic decreases in the hippocampus on GABA, CPP and on the CPP-parvalbin (PV) and parvalbin-calbindin (CB) in the hippocampus. Neurons were labeled by anterior staining at 38 hours by different times 100 minutes to 25 minutes. Adjacent sagittal 45 μm sections, obtained from injured animals were used to explore the distribution of the labelled neurons. The degree of decrease of GABA, PV and CB according to the NIBR-parvalbin immunohistochemistry. Animals exposed to methiothepin were used as control. The results obtained show a highly significant reduction of the number of GABAergic neurons in CA1, CA2, CA3 regions and in all of the dorsal hippocampus at the stages of the aged. Significant effects of neuronal axons as well as PV or CB neurons were evident. In the aged, as compared with the young group, a significant decrease in the number of neuronal axonal decrease in the CA1, CA2, CA3 sectors, while no statistically significant differences between the two groups were evident at P35 and P60. In spite of the effects of neuronal axons on the number of neuronal axions for GABA, PV or CB, the postmortem pattern of development of these neurons was similar in the two experimental groups. The developmental of both axonic and control rats GABAergic did not show significant modifications. PV-IR presented a progressive increase from P7 to P30, while CB-IR had a marked decrease with a decrease from P7 to P30 and a subsequent increase to P60. The present data demonstrate that, contrary to the data described after adult axonic, neuronal axonic induces the increase of the number of GABA of CA1 and CA3 hippocampal neurons while it affects only temporarily the PV-IR neuron density.

533.2
IMMUNOHISTOCHEMICAL OBSERVATIONS ON GABAERGIC NEURONS FOLLOWING CARDIAC ARREST CEREBRAL ISCHEMIA. K. Kawai, C. Rützler, I. Nitecki, L. John, and I. Klatzo*. Stroke Branch, NINDS, NIH, Bethesda, MD 20892

Description of characteristic, early changes affecting predominantly GABAergic neurons (J. Cereb. Metab., 12: 238-249, 1992), prompted the present study concerned with evaluation of changes in parvalbumin (PV) and glutamate decarboxylase (GAD), both proteins related to the GABA neurotransmitter. Observations with application of specific antibodies to these compounds revealed a marked reduction of both PV and GAD immunoreactivity in the neurons of the n. reticularis thalami (NRT), at 1 h-interval following ischemia. Staining with GAD at that time also showed an intense immunoreactivity of enlarged and seemingly degenerating terminals and boutons in the ventral thalamic nuclei, medial geniculate, inferior colliculus and other structures. During the first week after ischemic insult, the GABAergic terminals and boutons in these areas appeared inconspicuous or absent. After 2 weeks, there was an indication of the regrowth and sprouting of new neurons and terminals by GABAergic circuitry. Also, our study demonstrates a postischemic attempt at regeneration as shown by sprouting of presumably GABAergic terminals.

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GLUTAMATE RELEASE INDUCED BY OXYGEN/GLUCOSE DEPRIVATION IN CORTICAL CELL CULTURES. D. Lebeut* and D.W. Choi, Dept. of Neurology, Washington Univ. School of Med., St. Louis, MO 63110

Mature cortical cell cultures, containing both neurons and glia, exposed to combined oxygen-glucose deprivation for 45-75 min developed anoxia-sensitive swelling, and widespread neuronal degeneration by the next day. At termination of oxygen-glucose deprivation, an increase in bathing medium glutamate could be detected by HPLC using phosphonitriocyanate derivatization and UV detection: typically the glutamate concentration in the extracellular medium increased from about 200 μM to 2.3 μM. Assuming the glutamate originates from neuron and glia, >1000 neurons per 15 mm well containing 375 μL medium, this would correspond to the release of 2.3 X 10⁶ molecules of glutamate per neuron. Distributed in available neuronal cell body volume (mean neuronal cell body radius 7.8 microns), a concentration of 2 mM would result. Most likely, much of this glutamate originates from presynaptic terminals where original glutamate concentrations may be much higher. Both the increase in extracellular glutamate and neuronal death were attenuated by addition of 10 μM N-6-cyclohexyladenosine, an AI-adenosine receptor agonist, or 100 μM D-tubocurarine, to the cultures during the oxygen-glucose deprivation, consistent with the idea that the increase in extracellular glutamate is related to observed neuronal injury. While the measured increase may be too small to cause excitotoxic injury itself, higher glutamate concentrations may build up loco to synaptic release sites.

SODIUM REGULATES ANOXIA-INDUCED INTRACELLULAR CALCIUM CHANGES IN CA1 HIPPOCAMPAL NEURONS. J.E. Friedman* and G.G. Hedgpeth, Dept. Pediatrics, Section of Respiratory Medicine, Yale Univ.-Sch of Med., New Haven, CT 06520

Anoxia is believed to induce neuronal damage by causing a sustained increase in Ca²⁺, possibly via glutamate excitotoxicity. Using freshly dissociated rat hippocampal CA1 neurons, Ru-3 and microscopy, we have previously shown that anoxia causes a rapid (within 2 min) increase in Ca²⁺ accompanied by swelling, but that this increase is not glutamate mediated: in addition, neuronal injury does not have to be accompanied by an increase in Ca²⁺. To further understand the mechanisms underlying swelling and injury, we focused on the role of Na⁺. Upon replacing Na⁺ with N-Methyl-D-Glutamine (NMDG), Ca²⁺ increased, but anoxia caused a sharp decrease to below baseline levels. Neurons remained morphologically intact without swelling, for up to 90 minutes after anoxia. Adding Na⁺ at any time after anoxia resulted in re-osmolarization and injury. Addition of Beprlid (10μM), a Na⁺/Ca²⁺ exchange blocker, caused this same sequence of events to occur with NMDG, although the neurons did eventually swell following anoxia. EIPA (10μM), a Na⁺/H⁺ exchange blocker, had no effect. The Na⁺-channel blocker TTX (0μM) did not affect baseline fluorescence, but caused a rapid decline in Ca²⁺ after anoxia-induced transient rise. We conclude that (1) the increase in Ca²⁺ that occurs with anoxia is Na⁺-dependent, 2) anoxia-induced neuronal swelling is caused by Na⁺ entry and removal of Na⁺, 3) to a great extent Na⁺ does not enter through voltage-sensitive Na⁺ channels and 4) possible re-oxygenation injury requires the presence of Na⁺.


Brain intracellular pH (pHi) falls dramatically during the total cerebral ischemia that occurs during cardiac arrest. The level of acidosis depends upon the pre-arrest plasma glucose level and the amount of lactate accumulated during the arrest. Because brain hyperglycemia during the ischemia has been associated with poor recovery, tissue acidosis has been proposed as being responsible for tissue damage due to ischemia. The purpose of this study was to determine the time course of pHi and tissue lactate recovery and to find out if they are correlated in the postischemic recovery period.

Reversible cardiac arrest was produced in male Wistar rats (200-300g) by KCL administration, followed after 5-7 min by resuscitation by chest compression and mechanical ventilation. The pHi was determined in frozen rat brains by histophotometry of neutral red before cardiac arrest, at the end of the 7-10 min ischemic duration, and at 5, 15 and 60 minutes after successful resuscitation. Control brain cortical pHi was 7.13 ± 0.06 (SE, n=3). During ischemia, pHi fell below 6.3 and then returned to control values within 5 min of resuscitation (7.15 ± 0.03, n=3) and remained constant thereafter. This also occurred in rats made hyperglycemic before arrest. In both groups brain lactate levels after 5 min of resuscitation remained at ischemic levels. Reversal of acidosis was delayed by treatment with inhibitors of Na+/H+ exchange transport.

In conclusion, reversal of brain intracellular acidosis after resuscitation from cardiac arrest is rapid, and thus postischemic acidosis can play no continued role in tissue damage.

EFFECT OF (3H)-D-ASPARTATE FROM RAT HIPPOCAMPAL SLICES DURING IN VITRO ISCHEMIA IS NOT BLOCKED BY THE NA-COUPLED CARRIER INHIBITOR, DINITROBENZATE. V. Montague*, F.L. Lindsay, Dept. of Physical Medicine and Surgery, University of Wisconsin, Madison, WI 53706

Excess release of glutamate (GLU) very probably contributes to ischemic brain damage. The mechanism of ischemia-induced release was unknown but a suggested possibility was reversal of the Na-coupled high-affinity glutamate transport system. We tested this hypothesis using the transport substrate (3H)-D-ASP (D-aspartate) and the transporter inhibitor, dihydroxy (-)-phenylalanine (DPhenylalanine) completely blocked Na-coupled uptake of D-ASP. Slices were loaded with ATP and then exposed to in vitro ischemia (IVI, 0-glucose/0-equilibrated buffer/37°C) for 3 successive 5 minute periods within 5 minutes. D-ASP efflux increased x1, x2.5, and x3.6 in the three periods. These increases were unaffected by 20 min. pre-incubation in 0-Ca buffer. 500 μM DPH did not affect D-ASP efflux during control period or during IVI.

50 μM verapamil increased D-ASP efflux by x1, x1.7, and x2.3 during 3 successive 5 minute periods. DPH completely blocked this increase, showing DPH very probably blocks D-ASP release when it occurs via reversal of the coupled transporter. Thus, Ca-independent GLU release during ischemia does not appear to occur via reversal of the Na-coupled transporter. Release of 3H-GLU from slices that were not increased by IVI, indicating release does not result from membrane "leakiness". Other mechanisms are being investigated.
533.9


Pre-treatment of neonatal rats with dexamethasone prevents brain damage associated with hypoxia-ischemia (unilateral carotid occlusion +3 hr hypoxia) (Ped. Res. 38:553-63, 1991). We presently investigate whether hyperglycemia or an induction of endogenous free radical scavengers explains dexamethasone’s neuroprotective effect.

Pathological changes in brain are maintained hyperglycemic during hypoxia-ischemia by the administration of 10% dextrose (1 ml/p) at 0, 1, 2 and 3 hr of hypoxia (n = 14) and compared to that in control (n = 15) or dexamethasone (1 mg/kg, i.p. n = 15) treated animals. Despite similar elevations in blood glucose at the end of hypoxia, dextrose treated animals had greater damage than dexamethasone treated animals and both of these groups had less damage than controls (volumes of damage of approx. 32 ± 3 vs. 9.5 ± 3; 58 ± 7% 2 isplastically, respectively; p < 0.0001).

Antioxidant enzymes were measured within brains of animals treated with dexamethasone or vehicle (n = 44). Cerebral concentrations of catalase, glutathione peroxidase and CuZn or Mn superoxide dismutase were similar in both treatment groups, with or without exposure to hypoxia-ischemia.

Thus, the relative hyperglycemia associated with dexamethasone treatment may partially contribute to the protective effect of dexamethasone. However, an additional glucocorticoid mediated effect other than hyperglycemia, or a change in antioxidants, may also be involved in dexamethasone’s prevention of hypoxic-ischemic damage. (Supported by the Heart and Stroke Fndt of Ont (T0265) and MRC (PG-42).

533.10


Hep 72, one of the 70 kDa family of heat shock proteins, was measured in astrocyte cultures subjected to heating to 44°C for 40 minutes. Pretreatment with dexamethasone, 10^{-8} M increased hsp72 expression in the heated astrocytes with its peak effect at 10^{-7} M. Other steroids (cholesterol, estradiol, progesterone and aldosterone) had little effect. RU486, a glucocorticoid receptor antagonist, blocked the effect of dexamethasone on heat-induced protein synthesis, as measured by incorporation of [35S]-methionine into peroxisomal acid precipitable material and this effect was antagonized by dexamethasone but not by the other steroids. These results suggest that dexamethasone potentiated the heat-induced response in astrocytes by acting through the glucocorticoid receptor. The functional significance of this effect is being investigated.

533.11


The neuronal damage surviving the primary of brain ischemia is in part mediated by the persistent stimulation of excitotoxic glutamate receptors. It is therefore conceivable that the expression of these receptors may be modified in the process. In the phototoxic model, retinal damage (Watsen B.D. et al., An. Neurol. 17:497-504, 1991), we studied whether there was a change in ionotropic glutamate receptor expression in the focal and perifocal areas. The ionotropic glutamate receptor subunits were visualized immunohistochemically with antisera directed against GluR1, GluR2/3, GluR4 or by in situ hybridization using cRNA probes that detect mRNA’s for GluR1 and GluR2 and their alternatively spliced forms. In control brains the various antibodies and the two RNA probes yielded very unique cytonological and topographical profiles. Following focal ischemia and in an area immediately surrounding the foci of the lesion (i.e., perifocal area) GluR1 and GluR2/3 immunolabeled cells were dramatically reduced in number and in staining intensity as early as 6 hours post-focal ischemia. A similar rapid decrease was also observed for GluR1 and GluR2/3 mRNA. Importantly, in this perifocal area the relative early decrease in the expression of glutamate receptor subunits proteins and message expression probably precedes any neuronal loss. In fact, these neurons still maintain the ability to respond to a glutamate induced event as demonstrated by the increased expression of c-fos mRNA and FOS. In contrast, GluR4 immunoreactivity was not reduced but rather in several instances displayed elevated levels which also correspond temporally and topographically with the rise in vimentin-positive and glial fibryl protein acid-labeled elements both within the perifocal area and throughout the ipsilateral cortex. Importantly, the perifocal area is a region in which profound cellular reorganization occurs and is a key region to target various neuroprotective drugs.

534.1


We previously reported that the interval between the coherent discharges of different postsympathetic sympathetic nerves can be frequency dependent in the 8-12 Hz band. In the current study we consider the possibility that the frequency-orientation behavior is a property of a system of weakly coupled nonlinear oscillators. This model was tested by forcing the central system responsible for the generation of the discharge (SN) at a single or repetitive electrical stimuli applied to sympathetic neuromat sites in the medial lateral tegmental field (LTf) or sympathoinhibitory sites in the medial nuclei. The discharges were made simultaneously from the postsympathetic inferior cardiac, ventral, and renal nerves of baroreceptor-denervated, denervated intact and sham-operated rats. The neural discharges of the SN were phase locked and the SN oscillations were generated at 10 Hz in SN when periodic stimulation of frequencies between 8 and 12 Hz entrained the rhythm (phase locking ratio of one). Lower frequencies (<6 Hz) of medullary stimulation entrained the 10-Hz rhythm in SN to a harmonic of the stimulus frequency. Most importantly, the preferred phase locking ratio of 10-Hz discharges to stimulation of postsympathetic nerve could be different than that for a second nerve when the stimulus frequency was <6 Hz. On the basis of these observations, we propose that the circuits responsible for the neural oscillations in the SN were weakly coupled nonlinear oscillators, each of which either influences one postsympathetic nerve or nonuniformly affects different postsympathetic nerves. (Supported by NIH grants HL-13187 and HL-33266).

534.2


We have used spike-triggered averaging of sympathetic nerve discharge (SN) to identify RLVM and caudal medullary raphe nerves with activity correlated to both the 10-Hz and 2-6-Hz rhythms in referral to the corresponding nerve. By means of 2-6 Hz rhythms in SN to an harmonic of the stimulus frequency. Most importantly, the preferred phase locking ratio of 10-Hz discharges to stimulation of postsympathetic nerve could be different than that for a second nerve when the stimulus frequency was <6 Hz. On the basis of these observations, we propose that the circuits responsible for the neural oscillations in the SN were weakly coupled nonlinear oscillators, each of which either influences one postsympathetic nerve or nonuniformly affects different postsympathetic nerves. (Supported by NIH grants HL-13187 and HL-33266).
534.3 THE 2- TO 6-Hz RHYTHM IN SYMPATHETIC NERVE DISCHARGE (SN) IS AN EMERENT PROPERTY OF A BRAINSTEM NETWORK COMPOSED OF NONRHYTHMICALLY ACTIVE NEURONS. D.J. Gebber*, Z.S. Huang, S. Phong, and T.A. Miller. Dept Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

Individual medullary neurons with activity correlated to the 2- to 6-Hz rhythm in SN of baroreceptor-denervated cats have firing rates that generally are lower than the frequency of the rhythm recorded from sympathetic ganglionic neurons. These neurons fire on a single 2- to 6-Hz cycle of SN. Throttle rhythm in SN is not strictly represented in the spike trains of single medullary neurons. This suggests that each cycle of SN reflects synchronization of the discharges of larger neuronal populations comprising the brainstem network responsible for this rhythm. We tested this hypothesis in single neurons by the 2- to 6-Hz rhythm appears in population recordings (i.e., field potentials) made from the rostral ventrolateral medulla (n=6), medullary raphe (n=5), and lateral tegmental field (n=1). Autospectra revealed peaks in the 2- to 6-Hz band of activity recorded from these brainstem sites and the inferior cardiac and renal sympathetic nerves. The shapes of the autospectra of brainstem and visceral SN were indistinguishable. Coherent analysis demonstrated that the rhythms in brainstem activity and SN were significantly correlated. Peak coherence values were as high as 0.56. Cervical spinal cord transection only modestly reduced the power in brainstem population activity to 82% of control. Most importantly, the shape of the autospectrum of brainstem activity was unchanged. We conclude that the 2- to 6-Hz rhythm in SN arises from a network of brainstem neurons whose discharges are probabilistically related to the phases of the population rhythm. (Supported by NIH grants HL-13187 and HL-33266).


We have recently identified a vasodepressor area within the ventral and caudal region of the medullary reticular nucleus gigantocellularis, the giantocellular depressor area (GIDA), that is topographically distinct from other vasovagal sites in the medullary reticular formation (Acher & Reis, Neurosci. Abstracts, 1991). We sought to determine if efferents from the GIDA project to the intermediolateral cell column (IML) and lamina VII of the thoracic spinal cord and to characterize their synaptic relations with neurons in these areas. The anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) was injected into the GIDA of rats. In some cases, the GIDA was identified as a vasodepressor site by microinjection of glutamate into the site prior to the PHA-L injection. Ten days later, rats were anesthetized and perfused with acelone and paraformaldehyde. Sections of the thoracic spinal cord (T2) were processed for immunocytochemical localization of PHA-L. The injection sites were located and verified as being confined to the GIDA. PHA-L labeled punctate fibers were seen in the medial aspect of the IML and through the entire lamina VII. Electron microscopic analysis of the IML and lamina VII in these cases showed that PHA-L immunoreactivity was seen in myelinated and unmyelinated axons, as well as terminals (0.6-0.8 μm diameter). PHA-L labeled terminals contained small, clear vesicles and formed symmetric synaptic contacts primarily on large and small dendrites of neurons in the IML and lamina VII. These data demonstrate that neurons of the GIDA project directly to autonomic laminae of the thoracic spinal cord. Moreover, the present results indicate that this may be a direct and novel medullobulbar sympathoinhibitory pathway. (Supported by NIH HL-08051 & HL-18974).


Reticulospinal sympathoexcitatory (pacemaker) neurons of rostral ventrolateral medulla (RLVM) are rapidly excited by systemic hypoxia or by cyanide (CN) applied by microinjection and/or iontophoresis (Sun et al., J. Physiol. 382:R182, 1992). It is not known whether hypoxia/CN directly excites these neurons or if so by what mechanism. Electrophysiological studies were conducted in slices of rat medulla maintained in an interface-type chamber at 31°C. Pacemaker neurons were spontaneously active. Hypoxia (75% N2, 5% CO2, 8% O2) or CN (30-300 μM, 0.4% application) rapidly and substantially increased their activity. The activity of adjacent neurons was unaffected or reduced. The excilatory response to CN was dose-dependent, reversing reproducibly at 10 μM. It was characterized by membrane depolarization (21.4±4.2 mV with 300 μM CN) and an increase in membrane resistance (38±2.1 M Ω at 300 μM CN). The response persisted in the presence of tetrodotoxin (10 μM), which eliminated evoked and spontaneous action potentials indicating that hypoxic excitation was not a consequence of synaptic excitation. Under single electrode voltage clamp, CN evoked an inward current (peak: 0.58±0.08 nA) which was voltage-dependent and transient at membrane potentials between -70 and -40 mV (when stepped from -100 mV). The current was abolished by 2 mM CoCl2 or 100 μM NiCl2. We suggest that the RLVM pacemaker neurons are excited by hypoxia by increasing low voltage-activated calcium channel conductance. RLVM neurons may, like glomus cells of carotid body, be the targets for inhibiting the sympathoexcitation associated with cerebral ischemia.

534.4 ROLE OF THE SPINAL CORD (SC) IN GENERATING THE 2-4 Hz PEAK IN THE POWER SPECTRUM OF RAT SYMPATHETIC DISCHARGE (SN). A.M. Alford, J.M. Adams and P.G. Gyuret. Deps. of Pharmacology and Biomedical Sciences, Univ. of Virginia, Charlottesville, VA 22908.

The existence of a 2-4 Hz ‘throttle’ rhythm and fact that discharge of various types of medullary neurons is statistically correlated with this rhythm, have fueled arguments against the pacemaker theory of SN generation. The latter states that the basal SN firing is a result of the summed discharge of excitory premonitor neurons located in the rostral ventrolateral medulla (RLV PMNs). The main objection to the theory is that the discharge rate of these cells (3-35 Hz) is much faster than the peak frequency of the 2-4 Hz ‘throttle’ rhythm in rats (3-4 Hz). This objection rests on the assumption that the discharge rate and/or pattern of these cells is critical to the generation of the 2-4 Hz ‘throttle’ rhythm. The following data (in development, different unselectected rats suggest this assumption may be invalid. SN synchronization was determined by measuring the fractional power contained in the 2-4 Hz range of the SN power spectra from bipolar recordings of lumbar SN (3-1000 Hz recordings). Unilateral microinjection of muscimol (GABA agonist), or bilateral injection of a mixture of kynurenic acid (KYN, an excitatory amino acid) and bicuculline (GABA receptor antagonist), into RVL, produced a little effect on the 2-6 Hz rhythm. Intrathecal injection of KYN, at mid-thoracic levels slightly reduced the 2-4 Hz peak but SC transection at C8 had no effect, after SN had been restored close to control by intrathecal injection of kainic acid (EAAC receptor agonist). We conclude that i) the firing rate and pattern of the RVL input to the SC is not critical for production of a 2-4 Hz peak in SN power spectra, ii) in the rat this synchronization may be essentially of SC origin and iii) the existence of a 2-6 Hz rhythm of SN does not constitute an objection to the theory of SC generation. Support: HL 28785 from NIH and Medical Research Council of Australia.


The caudal ventrolateral medulla oblongata (CVL) contains depressor neurons whose activity tonically inhibits sympathetic vasoconstrictor tone, probably by inhibiting sympathoexcitatory neurons in the rostral ventrolateral medulla (RLVM). In urethane-anesthetized rats, catheterization between the CVL and RLVM was done. Perfusion timograms were constructed using an ITC16 interface and a Macintosh IIx computer programmed with ICGOR. We identified 134 spontaneously respirable neurons in the CVLM with projection to or through the RLVM. Twenty nine of 107 neurons tested (27%) were activated by increase in blood pressure, 60% were inhibited and 13% were unaffected. Injection of nicotine and/or aminophylline increased discharge of 29 neurons from 2 to 4 spikes/s to 6.5±1 spikes/s. Electrical stimulation of the ipsilateral aortic depressor nerve (3 pulses, 200 Hz, 0.5 ms) activated these neurons (latency 55±4 ms, conduction velocity 0.6 m/s). Neurons excited by an increase in blood pressure were located in the previously defined caudal vasodepressor region and also as far rostrally as 0.5 mm rostral to the obex. These neurons may form part of the central inhibitory link in the baroreceptor-pressor pathway. Other neurons may be inhibitory vasomotor cells with functions independent of baroreceptor inputs or they may be A1 catecholamine neurons with axons passing through the RLVM.
CARDIOVASCULAR REGULATION II  THURSDAY PM

534.9

CONNECTIONS BETWEEN PONTINE RETICULAR FORMATION AND ROSTRAL VENTROLATERAL MEDULLA IN CARDIOVASCULAR CONTROL. A.V. Kreiswitz* and L.C. Weaver. The John P. Robarts Research Institute and Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5K6.

A previous study in our laboratory established that the pontine reticular formation (PRF) is involved in the control of circulation (Haven et al, Neurosci. Abst.17: 706,1991). The connections between PRF and the well-known rostral ventrolateral medulla (RVLM) are undefined. To determine those connections, we investigated responses of single neurons in the rostral pontine reticular formation (RSNA), arterial pressure (AP) and heart rate (HR) to microinjection of the inhibitory amino acid glycine (1.0M, 60nl) into the PRF before and after unilateral or bilateral microinjection of the synaptic blocking agent cobalt (4.0M, 20nl) into the RVLM in propofol-anesthetized rats (n=19). Glycine injection into the PRF caused decreases in AP (-20±2 mmHg), HR (-5±1 bpm) and RSNA (-38±5%). Following microinjection of Co+ into the ipsilateral RVLM, responses to PRF blockade were significantly decreased: AP (+2±3 mmHg), HR (+420 bpm), RSNA (+6±2%). In contrast, injections of Co+ into the RVLM contralateral to the site of PRF blockade did not affect responses to this PRF blockade. Responses to PRF blockade after bilateral injection of Co+ into RVLM were similar to responses after unilateral injection of Co+. Somatosympathetic reflexes elicited in the renal nerve by electrical stimulation of the sciatic nerve were significantly inhibited after unilateral injection of Co+ into the RVLM and, after bilateral injection, they were abolished. The Co+ injections had no effect on basal firing of the sympathetic nerve or on arterial pressure or heart rate. These results suggest that the PRF influences on RSNA are relayed ipsilaterally from pont to RVLM to influence spinal sympathetic neurons. Supported by the Ontario Heart and Stroke Foundation.

534.11


Sympathetically related neurons have recently been identified in the lateral tegmental field (LT). A sub-population of these, the sympatheticoexcitatory (LT-E) neurons, have been shown to project to the RVLM and provide an excitatory input to the rostral ventrolateral medulla. We have demonstrated that these LT-E neurons are inhibited by 8-OH-DPAT and ischemic 8-OH-DPAT and 5-HT. The results of this study show that the inhibitory effects of 8-OH-DPAT on LT-E neuronal activity are mediated by excitation of 5-HT1 receptors. The results provide evidence for the involvement of sympathetic related neurons with the cardiovascular system.

534.12

CARDIOVASCULAR PROJECTIONS FROM THE AMYGDALA (AMG) TO THE BED NUCLEUS OF THE STRIA TERMINALIS (BST). S. Boder and J. Grell. Dept. of Physiology, Univ. of Western Ontario, Canada, N6A SC1.

Two series of experiments were performed to investigate the anatomy of the depressor responses elicited by electrical stimulation of AMG were mediated via projections to BST. In the first series, to determine whether AMG neurons innervate the cardiovascular region of BST, projections to BST from different subnucleus within AMG were investigated in the rat using the retrograde tracer Fluorescein (FG, 2%) and the anterograde tracer Phoxenosin vulgaris leucoagglutinin (PHAL, 2.5%). FG (50-10000) injections overlapping the cardiovascular region of BST resulted in retrogradely labelled neurons throughout the AMG. Lesions with PHAL was produced with small injections of the AMG (A3) and basolateral nucleus of the AMG (ABL) resulted in dense fiber and presumptive terminal labelling within the cardiovascular region of BST. In the second series, the effect of blocking synaptic transmission in BST with Co2+ or chemical lesions of BST with ibotenic acid (Sugul) on the depressor response elicited by stimulation AMG was investigated in the chloralose anaesthetized, artificially ventilized and paralyzed rat. Five mm microinjections of (200nl) of Co2+ into BST significantly reduced (41.2±7.3%) the depressor response during stimulation of A3. On the other hand, the depressor response to stimulation of ABL was significantly potentiated (57.0±16.6%) after Co2+ injections into BST. Microinjections of ibotenic acid (200nl) into BST resulted in similar effects on the depressor responses to AMG stimulation. Taken together, these results suggest that AMG neurons innervate cardiovascular regions of BST and that these BST neurons are a component of the neuronal circuit that mediates cardiovascular responses elicited during stimulation of AMG. (Supported by MRC and HSPO).

CALCIUM CHANNEL MODULATION

535.1

TWO VOLTAGE INDEPENDENT CALCIUM CHANNELS ARE ACTIVATED BY PERTUSSIS TOXIN IN PC12 CELLS. S.A. Scott* and I.A. Strong, Dept. of Biol. Sci., Purdue University, W. Lafayette, IN 47907.

Voltage independent calcium channels have been described in a variety of cell types. This broad class of Ca2+ channels includes channels that are regulated by either extracellular or intracellular ligands. We have observed two novel voltage-independent Ca2+ channels with different slow conductances (3ps and 16ps using 20mM Ca2+ as the charge carrier) in undifferentiated PC12 cells. These channels open rapidly during cell-associated recordings. The 3ps channel was activated by patch excision into a defined K-aspartate EGTA solution (n=13). Channel activity could be observed at very negative potentials (e.g., -110 mV). However, if the cells were pretreated with pertussis toxin (8-hours), both the small conductance Ca2+ channel and the larger conductance Ca2+ channel were active even in the absence of TPX (n=10). Like the 3ps channel, the 16ps channel was also seen over a wide range of potentials. Activity of both channels was observed at the cells' resting membrane potential. The effect of pertussis toxin on Ca2+ channels in PC12 cells is controlled by the control of a GTP-binding protein. The 3ps channel may be similar to the pertussis toxin activated Ca2+ channel reported by Cetha et al in bovine chromaffin cells. Preliminary results suggest that pertussis toxin activates the small conductance channel (n=3) via an intracellular pathway. This suggests that this channel may be responsible for the sustained Ca2+ influx seen in cells treated with bradykinin. A physiological activator of the 16ps channel has yet to be found.


535.2

SUBSTANCE P INHIBITS Ca2+ CURRENTS IN RAT SYMPATHETIC NEURONS VIA A PERTUSSIS-INSENSITIVE G-PROTEIN. M. S. Bhandari* and M. Hille, Dept. of Physiology and Biophysics, Univ. Washington, Seattle, WA 98195.

We studied inhibition of N-type Ca2+ channels in adult rat superior cervical ganglion (SCG) neurons by substance P (SP) using the patch clamp. In whole-cell configuration with external 5 mM Ca2+, 70 of 82 acutely dissociated SCG cells showed inhibition by 500 nM SP. Peak currents elicited by 12 ms voltage steps to 0 mV were reduced by 37% (2.2 mean±SE). Treatment of overnight SCG cultures with 500 nM/ or 100 nM/ peroxidise toxin (PTX) had no effect on SP inhibition compared to treatment with 500 nM/ heparin (n=7). Dialysis with 2 ml GDP-B-S for >7 min. reduced SP inhibition (to 16±4%, n=12) implicating G-proteins. Inhibition measured by tail currents in 400 nM/ heparin and 200 nM/ heparin (n=7) and 120 mM Ca2+ was not voltage dependent, and current activation was not slowed or biphasic, indicating a mechanism other than a "reluctant-willing" Ca2+ channel. In rat SCG neurons, 100 nM/ substance P (A=10) or 1 nM/ neurokinin B (13 of 14 cells) was without effect, suggesting NK1 but not NK2 or NK3 subtypes of tachykinin receptors. Intracellular Ca2+ levels were not significantly increased by SP inhibition with 120 mM BaCl2 in the pipette (48±8%, n=9) was only slightly greater with 200 mM BaCl2 (33±5%, n=9). Cell-attached patches with SP applied to the bath were sensitive to 120 mM BaCl2 in the pipette and 150 KCl/300 Ca in the bath, only 12 cells showed inhibition of currents in the patch upon addition of 500 nM SP. We conclude that SP inhibition in rat SCG neurons is insensitive, membrane delimited and may be mediated by the direct action of a PTX-insensitive G-protein on Ca2+ channels in SCG neurons.
535.5 ETHANOL SUPPRESSES NEURONAL CALCIUM CURRENTS BY G-Protein ACTIVATION. A. HERMANN, E. Lahnsteiner and H. Krebsbaum. University of Salzburg, Dept. of Physiology, Salzburg, Austria.

Recent research indicates that voltage- and ligand gated ion channels are involved in the action of ethanol. In mollusc neurones calcium currents are suppressed by ethanol (CAMACHO-NAVI and TREISTMAN, 1987), its mechanism of action is not understood. However, to study the action of ethanol in more detail we have used neurones in the central nervous system of the snail, Helix pomatia. Voltage clamp experiments show that ethanol (0.5%) suppresses calcium currents in a time- and voltage-dependent manner. Intracellular buffering of calcium using BAPTA abolished the ethanol effects. Employing confocal laser microscopy imaging techniques revealed that ethanol at low doses does not affect the intracellular calcium concentration. Injection of calcium into the cell after application of dopamine which also reduces calcium currents, had no further suppressing effect. Employment of CAM activators (BcAMP, bpmAMP) decreased the calcium current, indicating ethanol may act after a maximum effect was obtained. A block of calcium effects by taurine and phorbol esters (PDBu) recently observed in 1986, confirmed these findings. Furthermore, the results taken together suggest that the suppression of calcium currents by ethanol is caused by a G-protein - protein kinase C transduction pathway.

535.7 ENHANCEMENT OF NEURONAL N- AND L-TYPE Ca2+ CHANNEL ACTIVITY BY PROTEIN KINASE C. J. Yang and B. W. Tsien, Dept. of Mol. & Cell. Physiol, Univ. of Michigan, Ann Arbor, MI 48109

We studied effects of phorbol esters on Ca2+ channel activity in frog sympathetic neurones. High-voltage activated Ba2+ currents in whole-cell recordings were increased (27 ± 3% n=10) by 1 μM phorbol dibutyrate (PDBu), a stimulator of PKC. The inactive congener 4-α-phorbol produced no such increase (3 ± 2% n=10). The PDBu-induced block was blocked by the inclusion of the PKC-blocking peptide PKC-like (19-31) in the pipette (internal solution) (5 ± 4% n=4), or staurosporin in both the external and internal solutions (12 ± 2% n=6). These observations indicate that Ca2+ channel activity is increased by activation of PKC. Enhancement by PDBu was not caused by removal of tonic G-protein inhibition since it was observed in cells dialysed with 2 mM GDP-β-S (0.5% n=2). N-type currents (defined as resistant to 10 μM nimodipine susceptible to inhibition by ω-CgTx or norepinephrine) were increased by 31 ± 14% (n=5). Moreover, PDBu increased L-type tail currents made prominent by Bay K 8644 in the presence of ω-CgTx. Direct enhancement of L-type currents (resistant to 5 μM ω-CgTx but susceptible to block by nimodipine) was also enhanced (31 ± 14%, n=5). Furthermore, PDBu augmented L-type tail currents made prominent by Bay K 8644 in the presence of ω-CgTx.

Dramatic increases in unitary N- and L-type channel activity were seen in cell-attached patches following applications of PDBu to the bath of the cell. In most recordings, the control activity was of the low open probability (p_o) mode. PDBu did not affect the unitary current size or mean open time but increased p_o several-fold by sharply decreasing closing time intervals between adjacent openings. Enhancement of Ca2+ channel activity by PKC may be important for various neuronal functions such as synaptic plasticity and development.


We studied the actions of phorbol esters on Ca channels in rat cerebral cortex. Phorbol-12-myristate-13-acetate (PMA, 10 nM, n=10) increased Ca currents on CA1 neurones. First, high-voltage activated Ca channel current was enhanced (29 ± 3% of -40 mV, 12 cells). Enhancement typically began within 10 seconds of PMA application and reached steady state within 1-1.5 min. In most cells the enhancement was larger for small depolarizations; in one cell Ca channel enhancement was 49 % at -20 mV. 31 ± 0 % at 0 mV, 20 % at -20 mV and 12 ± 4 % at +40 mV. Second, the ability of metabolotropic glutamate receptor agonists to modulate Ca channel current is greatly reduced after application of PMA; 1S,3R-ACPD suppressed Ca channel current by 24 ± 4 % prior to, and 2 ± 0.4 % following PMA application. Both of these actions were blocked by inclusion of PKC-19-36 in the pipette (100 μM). Neither of these effects were seen after application of 4-α-CPA (8 M); in 10 control cells 10 μM ω-CgTx (GKVIA) blocked 17.4 ± 1.3 % of current while in 9 cells where PMA was applied 10 μM ω-CgTx-GVIA blocked 21.7 ± 3.5 % of current. In control neurones 10 μM ω-CgTx (GKVIA) reversibly blocked 10 μM Ca channel current; block by 10 μM ω-CgTx (GKVIA) occurred further block by 10 μM ω-CgTx-GVIA (4 M). In 4 cells 10 μM ω-CgTx-GVIA (MIVC) blocked 17.2 ± 2.4 % of current prior to, and 32.1 ± 2.4 % of current following PMA application. In contrast 10 μM nimodipine blocked 12.6 ± 2.5 % of current by 30 minutes following PMA. PMA also enhanced Ca channel current in rat sympathetic neurones (25 ± 2.7 %; n=9). Experiments with blockers in sympathetic neurones confirm the notion that PMA stimulates N-type Ca channels.

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535.9 Metabotropic glutamate receptor stimulation increases calcium currents in rat cerebellar granule cells. J. L. Bossa, L. Fagi, J. M. Nooney, J. Bockaert, and A. Felizuet. Neurobiol Cellulaire, 5 rue d'Aubervilliers, F-76070 Rouen, France, and Inserm U. 56, rue Blaise Pascal, F-63000 Clermont-Ferrand, France. 1991 Eur. J. Neurosci. 3, 778-789. The latter effect is sensitive to external Ca2+ which suggests that QP receptor stimulation may promote Ca influx. The effect of t-ACPD, a selective QP agonist, on Ca whole-cell and channel activity were studied on granule cells maintained in elevated K+ culture media for 5-14 days. 100 M t-ACPD increases both macroporous and unitary Ba currents in a percentage of cells. Both activities are sensitive to dihydropyridines. Application of t-ACPD to the cell body during cell-attached recordings indicate that a second messenger pathway is involved. t-ACPD increases single channel activity following pre-treatment of the cells with the membrane-permeable Ca chelator BAPTA-AM (50 M for 30 min). Moreover 100 M thapsigargin does not mimic the action of t-ACPD on Ca channels. Both observations suggest that intracellular Ca2+ is not the second messenger.

535.11 SELECTIVE ADENOSINE RECEPTOR ACTIVATION BLOCKS N-TYPE BUT POTENTIATES P-TYPE HIPPOCAMPAL CA CURRENT D.J. Murgos, M. E. Adams, and A. P. Fox. Dept Pharmacology, University of Chicago, Chicago, IL 60637; Dept Neurology, Univ Calif, Riverside, CA 92521. Adenosine, released in response to increased neuronal electrical activity or metabolic demand, has been shown to both decrease synaptic transmission (Ginsborg & Henri, J. Physiol., 1972; Dunwiddie & Hoffer, Br. J. Pharmac., 1980; Silinsky, J. Physiol., 1984) and to produce an excitatory response (Sakurai & Okada, J. Physiol. 1991) in hippocampal synapses. Previous reports have shown that adenosine or its analogues reduce Ca current in dorsal root ganglion and hippocampal neurons (Delphin et al., Proc. Natl. Acad. Sci., 1986; Madison et al., Biophys. J. 1987; Gross et al., J. Physiol., 1989). The effect of selectively activating adenosine receptors was examined on Ca channels from acutely isolated pyramidal neurons from the CA3 region of guinea pig hippocampus using the whole-cell voltage-clamp technique. Activation of A1 receptors inhibited primarily a-Convexity-sensitive N-type Ca current although a fraction of Ca current inhibited by A1 activation was not a-Convexity sensitive. In contrast, activation of A2 receptors resulted in significant potentiation of the a-Agonist-sensitive P-type but not N-type Ca current. This potentiation occurred via a Ca-dependent pathway and was blocked by inclusion in the patch pipette of WPIPT (10 M), a specific protein kinase A inhibitor. Conditions which augment Ca current have been implicated as an important component of synaptic transmission, excitotoxicity, and long-term potentiation. Because of the ubiquity of adenosine, the differential effects of receptor subtype activation on Ca channels may have significant implications for neuronal excitability.

536.1 FASCICLUSION IV: STRUCTURE, EXPRESSION, AND FUNCTION DURING GROWTH CONE GUIDANCE IN GRASSHOPPER. A. Kasciski, D. J. Mathews, T. P. Nunn, and D. A. Bogue. J. Physiol., Dept. of Molecular and Cell Biology, U. of California, Berkeley, CA 94720. Three different surface glycoproteins were previously identified (fasciocin I, II, and III) that are expressed on subsets of axons/dendrites during embryonic development. To identify additional pathway recognition molecules, we used the 68F18 MAb to study the structure, expression, and function of fasciocin IV in the grasshopper embryo. Fasciocin IV is initially expressed in the embryonic CNS on the U longitudinal pathways, on the commissural bifurcations of the MP4, S, and 6 prophy, on a set of anterior axon tracts in the segmental nerve root, and on axons in the intersegmental nerve root. Fasiciation is also expressed in circumferential stripes on the surface of epithelial cells in the developing limb bud. The boundaries of these stripes correspond to the limb segment boundaries and, in particular, to the precise location of the growth cones of the hindlimb bud pioneer neurons (A1 neurons) make a characteristic ventral turn during their extension to the CNS. Embryos cultured in the presence of 68F18 MAb during Ti amon migration exhibit aberrant formation of this pioneer pathway specifically at the trocartenotax/cox segment boundary, where normally both fasciocin IV is expressed and the Ti axon turn ventrally. Based on protein sequence data, oligo-nucleotides were used for both PCR and library screens to isolate cDNA clones encoding fasciocin IV. Conceptual translation of these cDNAs suggests that fasciocin IV is an integral membrane protein with a signal sequence, a long extracellular domain, a transmembrane domain, and a short cytoplasmic domain. The extracellular domain has three clusters of repeats (16 in total). No homologies have been found between fasciocin IV and any other proteins in the available data base. We are looking for the fasciocin IV homologue in Drosophila.

536.2 AXONAL PROJECTION PATTERN ARE ALTERED IN A NEW DROSOPHILA MUTANT, midline uncoordinated (muc). R. K. Murphy* and Randall W. Phillips. Neurobiology of Development Program and the Department of Zoology, University of Massachusetts, Amherst, MA 01003. Our objective was to uncover new mutations that alter the neural circuitry of the thoracic nervous system of flies. Newly hatched muc flies were isolated in a F2 screen of lines outcrossed with the controlled mobilization of a P element (P[lacW]). Bier et al. 1989. Mutant flies were identified in a screen for abnormal grooming behavior, mutants did not remove dust from their bodies as efficiently as wildtype. In one of these mutants (muc) the legs are brought into the proper position at the midline, rubbing movements occur, but the legs do not touch at the midline and dust particles do not collect there. This effect is consistent with changes in the axonal pattern of movements along the midline led us to examine sensory neurons whose axons normally cross the midline in the CNS and whose target is the muscle innervation. The axon projection pattern of the large tactile hairs called ASC and PSC in mutant flies lack the axonal branch contralateral to the cell body. This branch is present in 100% of the wildtype flies but was reduced or missing in less than 50% of wildtype flies. When the P element was excised from the second chromosome, both the behavioral and the axonal pattern returned to wildtype, confirming that the defect was caused by the insert. Analysis of the lacZ reporter gene shows that the posterior peripheral nervous system developed normally in the mutant. The sensory motor neurons of the midline were not affected. A variety of cells in the central nervous system are still affected and analysis of these cells may suggest that the mutation is causing a defect in the assembly of the midline. Supported by NIH grant NS15571 to R.K.M.
536.3


In order to elucidate the molecular mechanisms that guide the growth cones that pioneer the longitudinal and commissural axon pathways in the developing CNS of the Drosophila embryo, we performed a large-scale F2 genetic screen to identify mutations that disrupt the formation of these pathways. Approx. 13,000 independent lines, with ~3 lethals per chromosome, were screened for mutants that produce specific axons in the major CNS pathways as recognized by MAb BP102. Last year we reported on mutations in commissural pathways which perturb the commissural pathways (Seeger et al., 1991). Here we report on mutations which perturb the longitudinal pathways. We saved ~250 mutant lines, and separated them into several discrete phenotypic groups; some appear to have normal pattern formation and cell fate decisions, but show specific guidance defects. Mutations in one gene, longitudinal lacking (lak), lack most longitudinal tracts while having near normal commissures, peripheral nerves, muscles, and body organization. The neurons that pioneer the MP1 pathway project their growth cones correctly to their normal targets; however, they often stall and fail to form the pathway. The longitudinal glia are born, initially migrate, and divide as normal; the earliest defect is seen about the time that the pioneering growth cones contact these glia and fail to extend along them. A mutation in a second gene, roundabout (rob), leads to a dramatic missorting of the MP1 pathway. Defects are seen in a number of pioneering growth cones, including the MP11 growth cone, which extends posteriorly, but at the anterior commissure of the next posterior segment, makes a 90° turn towards the midline where it often contacts its contralateral homologue. The MP1 and other axons then form large circles of axons around the midline of two adjacent segments. We believe that these genes and others under investigation may play a role in the guidance of pioneer growth cones. The cloning of several of these genes is currently underway.

536.4

GENETIC ANALYSIS OF MOTONEURON PATHFINDING AND NEUROMUSCULAR CONNECTIVITY. D. Van Vactor and C.S. Goodman. HHMI, Dept. of Molecular and Cell Biology, U. C., Berkeley, CA 94720

The ability of motoneuron growth cones to find and recognize their correct muscles has been a model system for studies on the mechanisms of target recognition; in both vertebrates and invertebrates, motoneuron growth cones extend toward and innervate the appropriate muscle in a highly stereotyped fashion. Neuromuscular specificity in Drosophila is particularly well suited for studies in vertebrates because of the simplicity and target recognition. The body wall musculature of Drosophila embryos and larvae consists of 31 individually identified muscle fibers in each abdominal hemisegment. Most if not all of these targets are innervated by one or only a few motoneurons. Extensive analysis from several labs of normal and mutant embryos in both grasshopper and Drosophila argue for a high degree of specificity in the ability of motoneuron growth cones to recognize particular muscle fibers. Monocular antibody TD4 directed against the cytoplasmic domain of fasciclin II specifically labels a large subset of motoneuron growth cones and axons (but not sensory neurons) in the periphery. We have used this and other probes to characterize the specific pathfinding of the aCC, three U's, and RP2 growth cones as they extend from the ventral nerve cord towards the most dorsal muscle fibers. In order to gain insights into the molecular mechanisms underlying pathway and target specificity, we have embarked upon a large-scale mutagenesis screen to identify the pattern of neuromuscular connectivity directly in whole-muscle embryos. Our goal is to nearly saturate the Drosophila genome for mutations that produce specific defects in the innervation of the aCC muscles and motoneurons. Even though the screen is still in its infancy, we have already isolated a number of new mutants that perturb the projection of motoneuron growth cones.

536.5


Receptor-linked protein tyrosine phosphatases (PTPases) often contain ankyrin-receptor-like extracellular domains, and may thus couple cell recognition to signal transduction via control of tyrosine phosphorylation. We previously showed that three adhesion molecule-like PTPases are selectively expressed on CNS axons during the period of axon outgrowth. We have now found that another Drosophila pTPase, DPTPh1, is expressed primarily on CNS axons. DPTPh1, which was previously sequenced, contains two immunoglobulin-like domains and two fibronectin type III (FN) domains. To define the roles these PTPases play in neural development, we are making mutations in the growth cone, which extends posteriorly, but at the anterior commissure of the next posterior segment, makes a 90° turn towards the midline where it often contacts its contralateral homologue. The MP1 and other axons then form large circles of axons around the midline of two adjacent segments. We believe that these genes and others under investigation may play a role in the guidance of pioneer growth cones. The cloning of several of these genes is currently underway. We have developed a 'local transposition' method for isolating insertions of P-element transposons at specific locations, and have been able to use this method to make mutations in the DPTPh1 gene. We have shown that both DPTPh1A and DPTPh1D do not act as homophilic adhesion molecules in transfected tissue culture cells, suggesting that if they are involved in cell recognition they must recognize either in a ligand or growth cone.

536.6

GUIDANCE OF MOTONEURON OUTGROWTH BY A SERIES OF PERIPHERAL CUES DURING DEVELOPMENT OF DROSOPHILA. L.S. Wang* and H. Keshishian. Dept. of Biology, Yale University, New Haven, CT 06511

During neural development of Drosophila, axons of motoneurons follow stereotypic pathways and innervate specific body wall muscle fibers. Muscles 5 and 8 are innervated by a group of up to 4 motoneurons whose axons (here termed group 1 axons) form part of the efferent component of segmental nerve A (SNa). Other motor axons (group 2 axons) in the SNa make connections with transverse muscle fibers. It was shown previously that group 1 axons are capable of reaching the normal innervation region in the absence of their target muscle fibers 5 and 9 (Cash et al., J. Neurosci. 1992). In press). Our study of the temporal and spatial development of SNa shows that efferent axons first extend in the anterior half of the segment along the presynaptic axons of the ventral commissure sensilla 4 and 5 (vc4 and vs5). The two groups of motor axons contact the cell bodies of vc5 and branch at the lateral edge of muscle fiber 12 where group 1 axons turn posteriorly and ventrally to make contact with muscle fibers 5 and 8. Laser ablation of muscle fiber 12 results in the early posterior turning of both group of axons. Ectopic endings on muscle fiber 4 by group 2 axons are also observed. In the absence of both muscle fibers 12 and 5, delayed branching and turning of the group 1 axons are seen. We propose that the outgrowth of the motor axons of the SNa is guided by a series of peripheral cues provided by axon-afferent, sensory cell bodies, and both intermediate and target muscle fibers.

536.7

DOUBLE MUTATION OF FASCIICLIN I AND FASCIICLIN III CAUSES ERRONEOUS GROWTH CONE BEHAVIOR OF DROSOPHILA EMBRYONIC MOTONEURONES. A. Craie, T. N. Chang, and H. Keshishian. Dept. of Biology, Yale Univ., New Haven, CT 06511

The membrane proteins fasciclin I (fas I) and fasciclin III (fas III) are expressed by overlapping populations of Drosophila embryonic neurons in spatio-temporally specific manners. The two molecules do not interact directly in vitro and do not show possible involvement during axon pathfinding and synaptic targeting, we have examined null mutants of fas I and/or fas III using immunocytochemistry and intracellular dye-injections. Although the CNS of these viable mutants appears normal at a gross level, upon closer examination some identified motoneurons demonstrated consistent errors when both fas I and fas III were missing. Within the embryonic CNS the axons of motoneurons RP1, RP3, and RP4, revealed by antibody 2C10, repeatedly failed to maintain tight fasciculation within the normal pathways. The motoneuron axons in the periphery, labeled by neuron-specific (Neuro-GFP) antibody, showed apparent bipolar spreading during synaptogenesis. These observations were further confirmed by dye-injections into the RP motoneurons. The motor endings in the mature (third instar) larvae sometimes exhibited anomalous branch extensions. In contrast to the neuronal responses, the axons of either protein alone had no detectable differences from wild type. The results suggest that a subset of Drosophila embryonic growth cones may respond to at least two functionally redundant signals during their pathfinding and synaptic targeting.

536.8

HIERARCHICAL GUIDANCE CUES AND THE FORMATION OF MOLECULARLY DISTINCT AXONAL TRACTS DURING LEECH DEVELOPMENT. K. E. Joab, W. Mill, W. Reiter, and J. Johansson, Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011

In the CNS of the leech, the central projections of peripheral sensory neurons during early development segregate into three distinct axonal tracts, which are labeled by the monoclonal antibody lan-3-2 (Joab et al., J. Neurosci. 8:559, 1992). We have also shown that a subset of these neurons, recognized by the lan 4-2, projects its axons selectively into only one of these fascicles. Here we report on a molecular and developmental characterization of another fascicle specific antigen recognized by the monoclonal antibody lan-3-6. We have now found that this is the case with the lan-4-2 epitope the lan-3-6 epitope is only expressed by a subset of the peripheral neurons. The axons of these neurons also show selective fasciculation in the CNS, but only to a single one of the three lan-3-2 positive tracts, which, very interestingly, is a different one from the lan-4-2 positive tract. Thus, these observations provide further evidence for the existence of a hierarchy of guidance cues mediating specific tract formation in this system. We have screened an expression vector library with the lan-3-6 antibody and pulled out several potential lan-3-6 positive clones. Partial sequencing of one of these clones have revealed a protein with homology to proteins with EF-hand Ca-binding domains. However, from this general domain homology the sequence obtained appears to be unique. Immunoprecipitations followed by SDS-PAGE and silver-staining suggests that the antigen has a molecular weight of 150,000. Experiments with Northern and in situ hybridizations with the isolated clone are in progress in order to verify that the identified clones are indeed corresponding to the lan-3-6 antigen. Supported by NIH grant NS 28657.
536.9 IN VITRO STUDIES ON THE TOPOGRAPHIC PROJECTION OF NASAL RETINAL FIBERS ONTO THE CHICK POSTERIOR TECTUM. Yaender v. Boxberg, Silvia Deis, and Uli Schwarze*. Max Planck-Institut für Entwicklungsbiologie, Tübingen, P. R. G.

The stripe assay has been introduced as a model system to study the projection of retinal fibers from embryonic chicken onto their topographically appropriate regions on the tectum (Walter et al., 1090 Dev. 101, 909-913). By combining it with a novel membrane protein fractionation method we have been able to demonstrate that not only the temporal but also the nasal branch of nasotemporal guiding cues present on cell membranes derived from their proper target area. In addition, we could show that the survival of nasotemporal fibers in vitro can be substantially increased by and increased from the tectal system with membrane preparations from posterior tectum. We therefore suggest that trophic as well as trophic interactions may be involved in the Homer process of nasal axons within the posterior tectum.

536.11 CUTANEOUS AND MUSCLE AFFERENTS: INTERACTIONS WITH POTENTIAL TARGET TISSUES IN VITRO. S. A. Scott*, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

During embryonic development, cutaneous and muscle afferents appear to use different cues as they grow toward their respective targets. To test whether cutaneous and muscle afferents also differ in their response to the targets themselves, explants of the trigeminal mesencephalic nucleus (TMN), which contains only muscle afferents, or the dorsomedial pole of the trigeminal ganglion (DM-TG), which is largely cutaneous, from E10 chick embryos were co-cultured with either muscle or dorsal roots from E7 embryos. Interactions of individual growth cones with these potential targets were followed with time lapse video microscopy. In vivo few cutaneous axons penetrate the epidermis and most terminate in the dermis. Interactions of growth cones with myotubes, the target of muscle afferents in vivo, are also being investigated. Neither DM-TG nor TMN growth cones advanced onto epidermal explants, but over half of the TMN neurites remained in contact with the epidermis for > 1 hr. In contrast, many (10/19) DM-TG growth cones collapsed and retracted within 15 min of touching epidermis, and very few (1/19) maintained contact for > 1 hr. The behavior of both types of neurons was different upon encountering dermis. The majority (9/14) of DM-TG growth cones grew readily across dermis at rates comparable to their growth on the polyornithina-laminin substrate. In contrast, growth cones of TMN neurites did not advance well on dermis, and most (5/6) subsequently retracted to the substrate. Thus, there appear to be differences in the behavior of growth cones of E10 trigeminal cutaneous and muscle afferents. These differences could play a role in the selection of targets or could instead be a consequence of target innervation. Additional studies involving younger neurons that have not yet innervated targets will investigate these possibilities. (Supported by NIH grant NS-50697)


Developing retinal ganglion cell axons arriving at the ventral diencephalon establish an "X" shaped intersection of axonal projections from the two eyes seen as the optic chiasm in vivo. Thus it is surprising that axons from specific retinal regions sort into the correct optic tract. We previously found that the region of the future chiasm, prior to arrival of retinal axons, contains a population of neurons arranged in an inverted "Y" formation straddling the midline; and suggested that they may play a role in axonal guidance and formation of the optic chiasm.

We have now injected a monoclonal antibody (Mab) recognizing a 90kda cell surface molecule selectively expressed on these early chiasm neurons which is capable of labeling these neurons in live embryos. Injection of this Mab through openings made in the optic chiasm of E16 ventricles of mouse embryos at E11-E11.5, prior to arrival of retinal axons, ablates early chiasm neurons within 24 hours. Examination of injected embryos subsequently grown to hatching show that the "X" shaped optic chiasm which is normally present by this age is not observed. Axons in injected embryos have grown from both retinas into the optic nerves, but have failed to enter the diencephalon to reach the midline and form optic tracts. The formation of the optic chiasm was not prevented in animals treated with either complement or Mab alone or with a control Mab combined with complement. These results indicate that early ventral diencephalon neurones play a crucial role in the guidance of retinal axons and chiasm formation in the embryonic diencephalon.

536.12 THE LAMINATION OF HIPPOCAMPAL AFFERENTS IS NOT CAUSED BY THEIR SEQUENTIAL ARRIVAL DURING ONTOGENIC DEVELOPMENT. B. Heimrich and M. Preuschoft*, Inst. Anat., Univ. Freiburg, D-7800 Freiburg, FRG.

Afferents to the hippocampal formation are known to terminate in a laminated fashion. Bayer and Altman (1987) have provided evidence that the lamination of hippocampal afferents correlates with the neurogenetic gradient between cells providing input to the hippocampus. Thus, afferents from progressively later originating cells terminate progressively closer to the cell bodies of pyramidal neurons in the granule cells. Early arriving entorhinal fibers meet the yet short dendrites of the hippocampal target neurons. With further dendritic growth the entorhinal fibers eventually terminate in the most peripheral dendritic segments, whereas later arriving hippocampal (associational and commissural) fibers impinge on proximal dendritic portions. We have tested this temporal lamination hypothesis of hippocampal laminarization in an in vitro system. Two slices of hippocampus were co-cultured. Seven days later a slice of the entorhinal cortex was added to the two hippocampal slices. Fiber connections between the cultures were then labeled by using biocytin as an anterograde tracer. In contrast to the normal situation in vivo, the entorhinal fibers arrived later than the hippocampal fibers in this experimental paradigm. However, like in vivo, the entorhinal fibers terminated on the most peripheral dendritic segments of the co-cultured hippocampal target cells. We conclude that the sequential arrival of hippocampal afferents does not determine their termination in distinct layers.


(Supported by the DFG; SF5 325)

537.1 CATS WITH STRIATE CORTEX LESIONS CAN DETECT FINE GRATINGS BUT CANNOT IDENTIFY THEIR ORIENTATION. T. Packard and C. C. Olson*, Dept. of Neurobiology and Anatomy, Univ. of Rochester, Rochester, N.Y. 14642.

In the cat, areas 17 and 18 receive largely separate and distinct inputs from the lateral geniculate nucleus, the X-cells and the Y-cells respectively. Similarly, in X-cells, neurons have preferred spatial frequencies that are about three times higher than neurons in area 18. In this study, we examined the contribution of area 17 to visual function in two cats with fixation controlled by means of a retinal scotoma in the left visual field. We have identified neurons in the left visual field of area 17. Since neurons in this area respond to higher spatial frequencies than those in area 18, we expected to find deficit in area 17 and an increased spatial frequency in area 18. Initially, contrast sensitivity and visual acuity for the intact and the lesioned hemifields were measured in a detection task in which the cat indicated the presence or the absence of a vertical grating. With this task, sensitivity loss increased with spatial frequencies and then decreased again at higher spatial frequencies, with no loss in spatial resolution. The preserved sensitivity to high frequencies appeared inconsistent with loss of area 17 neurons. To examine the possibility that the preserved sensitivity to high frequencies was due to non-visual stimuli containing high spatial frequencies. The apparent preservation of sensitivity to fine gratings suggested by the detection performance may be due to signals from Y-cells which respond non-linearly to frequencies that are as high as those carried by linear X-cells in area 17.

(Supported by EY06175, EY03159, NS 27287)


This study measured the detection and discrimination performance of macaques, tested with controlled fixation, after localized cortical lesions produced by multiple injections of kainic acid. Primary visual cortex, area V1, is the target of most cortical afferents and is the most sensitive area that under most conditions, lesions of this area result in blindness. A V1 lesion of about 6 by 6 mm caused a 1.5 degree ofipsilateral scotomas and a greater deficit in performance of tests, which persisted throughout the 10 month post lesion test period. Cortical area V2 receives a substantial portion of the output from V1, although there are also pathways from V3, MT and other areas. Discriminative and chronic and luminance contrast sensitivity were measured by testing discrimination between gratings of horizontal and vertical orientations. The frequencies where most of the gratings were in which the cats discriminated between visual and horizontal gratings. With this task, sensitivity loss was minimal at low spatial frequencies and increased drastically at higher spatial frequencies. Visual acuity was reduced by about an octave. These results suggest that, in the cat, area 17 is critical to the vertical perception of stimuli containing high spatial frequencies. The apparent preservation of sensitivity to fine gratings suggested by the detection performance may be due to signals from Y-cells which respond non-linearly to frequencies that are as high as those carried by linear X-cells in area 17.

(Supported by AFOFSR 890041, and EY08988)

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THURSDAY PM
537.3

SELECTIVITY FOR FIGURE-GROUND DIRECTION AT OCCLUDING CONTOURS IN MONKEY AREA V2. E. Peterhans and R. von der Heydt, Department of Neurology, University Hospital Zurich, CH-8091 Zurich, Switzerland.

Visual processing of spatial information involves the detection of occluding contours and the distinction between foreground and background. We studied the neural mechanisms of such processes using the alert monkey. In area V2 we recorded the responses of single neurons to light and dark edges and to stimuli that mimicked situations of spatial occlusion, for example a light rectangle overlapping agrating of dark lines, or the same figure in reversed contrast. In neurons responding best to a group of such rectangles we studied the effects of figure-ground direction and contrast polarity.

About half of the neurons studied (19/40) were selective for figure-ground direction. However, these cells' selectivity was independent of contrast polarity, 10 cells showed interaction between these preferred stimulus attributes and the figure-ground direction. These findings can be interpreted in terms of a previously proposed model assuming that the figure-ground direction is inferred by asymmetric end-stopped cells (see TINS 14:112-119, 1991).

We conclude that mechanisms for the segregation of figure and ground are present at the level of area V2. Supported by SNF-grant 31-31970.91.

537.4


We have studied the responses of neurons in area V1 of anesthetized macaque monkeys to oriented texture elements that were part of a large texture field containing a texture border across which the element orientations differed by 90 degrees. The texture border was either matched or was orthogonal to the preferred orientation. Out of 161 cells tested, about one third showed a significantly greater response when the element was adjacent to the receptive field than when the texture border was moved. In most instances, the border enhancement was evident for only a subset of the border orientations tested. 10-15% of the total population showed a significant border enhancement for all orientations. We also tested for an orientation contrast (popout) effect: the responses to an oriented texture element were determined for an isolated element, an element embedded within a uniform texture field, and a single element within a field of elements of the orthogonal orientation. There was a clear, but imperfect, correlation between the two tests; many cells showing a border enhancement effect also showed a differential effect in the single-element popout test.

Altogether, these data support the hypothesis (derived from psychophysical studies) that texture segmentation is based on local feature contrast rather than on feature analysis extending over large texture regions (Nobisfurst, Vis. Res., 1991).

537.5


Human observers and monkeys can perceive the orientation of a boundary defined by differences in the direction of motion of two random dot fields, even if the fields are presented in opposite directions. Their orientation selectivity suggests an early processing stage for the detection of boundaries. We tested V2 cells with contrast luminance gratings and edges as well as kinetic boundaries generated using random dot fields moving in the same or opposite directions. Their preferred orientation was mapped, to enable us to align the center of the display with the boundary. Results show that MT cells of macaca fascicularis were not selective for the orientation of such kinetic boundaries. We have now extended this investigation to V1 cells.

We tested V2 cells with contrast luminance gratings and edges as well as kinetic boundaries generated using two random dot fields moving in the same or opposite directions. Their preferred orientation was mapped, to enable us to align the center of the display with the boundary. Results show that MT cells of macaca fascicularis were not selective for the orientation of such kinetic boundaries. We have now extended this investigation to V1 cells.

537.6


Usually, the strongest response of a visual cortical cell is obtained when the receptive field is crossed with an oriented moving stimulus. Recently, however, we have shown that small dots moving along the long axis of the r.f. elicit equally strong reactions in 80% of the cells (axial component; Exp. Brain Res. 76, 307-314, 1989). With small bar stimuli tuning curves with 4 peaks occur because of the axially directed orientation and axial components overlap. Strong axial responses are also obtained during repetitive stimulation with long bars using a grating with low spatial frequency. This observation made in 43 single units in area V1 in 17 of the anesthetized cat at moderate to fast temporal frequencies. In 8 cells the axial component was substantially stronger than the orientational component. Covering the OFF surround with a dark mask strongly enhances the axial component while diminishing the orientational component in cells with a clear ON-OFF midget separation. In these cases the cell's preferred direction turns by 90°. The "barber-pole" phenomenon is described by the effect that an edge will interact with motion perception. While viewing a moving grating partially obscured by an elongated edge a human observer will in most cases perceive motion along the edge regardless of the actual angle at which the grating is moving. The above described masking effect which shifts the preferred direction of the cells by 90° could be involved in this percept. The r.f.s distant from the edge will all be stimulated identically by the moving grating which will lead to adaptation effects. Thus, they should add little to the precept. On the other hand, t.f.s overlapping the edge are partly obscured and produce strong responses along the r.f. long axis which is parallel to the axis of perceived motion.

537.7


Principal component and information analyses (Optican and Richmond 1988, J. Neurosci. 8: 172-178) performed on spike trains recorded from single neurons in the temporal sensory areas of macaques performing a visual fixation task during the presentation of static face and non-face stimuli. Trans. Roy. Soc. Londo. (1992, 247: 11-21). We subtracted a correction from the calculated information to allow for the small number of trials, and showed there was a first principal component, which was highly correlated with firing rate, reflected most of the information available from the spike trains. We then showed that the information about the stimulus derived from the firing rate in a short period of 50 ms near the start of the elicited spike train was 0.57 of that which could be obtained using temporal encoding during the whole period. In a period of 20 ms, 0.43 of the information was available. When visual recognition of objects is performed by the visual cortex, there is a time delay only about 20 ms of processing within each cortical area before the next area must start its processing (Rolls, 1985, Current Opinion in Neurobiology 1: 274-278). The present results show that a significant proportion of the total available information is present within such short periods of the spike trains of neurons in so many systems. These results are consistent with the hypothesis that information from each cortical area is extracted from a short estimate of neuronal firing, rather than a longer estimate which allows temporal encoding to be analysed.
537.9
SPECTRAL SENSITIVITY FOLLOWING CORTICAL HEMISPHERECTOMY IN MAN. P. Stoerig1, L. Faehni2, M. Pitsis1, P. Lepor2, and A. Pits1. Inst. Medical Psychology, Munich University, FRG, School of Optometry2, Dept. of Psychology, Universität de Montréal and Montreal Neurological Institute3, Montreal, Canada. Increment-threshold spectral sensitivity was measured in the normal and blind visual hemifields of three patients who had undergone hemispherectomy 1.5-10 years previously. Under photopic and scotopic conditions with the patients monocularly fixating a fixation spot 17° eccentric from the center of the 90° field stimulated with 1000 nm light. The monitor screen around the stimulus was covered with a mask, and eye movements were monitored with an IScan Eye Movements Monitoring System. Stimuli with the same spatial wavelength from 450-610 nm were presented in random alternation with blanks, and their luminance was increased until the patients' detector reached the 80% correct criterion. The resultant spectral sensitivity curves show that the patients have a normal Purkinje-shift in both hemifields, although sensitivity in the blind field is reduced by 2 to 3 log units. Under photopic conditions, the curves exhibit the discontinuities attributed to colour-opponent interactions, showing that colour-opponent channels subserve the blind field even in the absence of one hemisphere. Unless the patients have abnormal retinal connections to the other hemisphere, this indicates that as yet unknown subcortical colour-opponent pathways survive the removal of a hemisphere with its massive degenerative consequences.

Supported by the Deutshe Forschungsgeemenschaft (STO 206-4) and NSERC.

537.11
DEPTH AND STIMULATION SITE OF MAGNETICALLY INDUCED PHOSPHENES IN THE BRAIN. D. P. Budgek and E. Mant. School of Optometry, University of California, Berkeley, CA 94720.

Magnetic phosphens were induced in normals with magnetic stimulator coils over the posterior pole. Peripheral phosphens (5°-50°) along the horizontal meridian are much more common than central ones (<5°) or along the vertical. This pattern is puzzling since (1) the central representation in visual cortex is much closer to the coil, and (2) there is no obvious reason why one meridian should be favored. Also, when we measured the stimulation depth using 2.5 to 5.0 cm from the midline, or -2.5 cm below the cortical surface at the pole, regardless of whether the phosphene was central or peripheral. Thus, there seems to be a lowered threshold site for stimulation deep in the occipital lobe, like that found in the ventricles. Such sites, e.g., should lie at sharp bends in the nerve fibers or large tissue conduction changes. We postulate that our site lies near the tip of the posterior horn of the lateral ventricle, because it has: (1) a large conduction change between it and adjacent white matter, (2) about the same depth as our site, (3) the optic radiation fibers surrounding it laterally, the horizontal meridian fibers being adjacent and the vertical fibers more distant, (4) peripherally projecting radiation fibers roughly parallel to the nerve fibers (i.e., central fibers are oblique), and (5) the calcarine sulcus (representing the peripheral hom. merd. in V1) medial and adjacent. This may explain why phosphens are more common in the horizontal meridian. Fiber tracts adjacent to ventricles elsewhere in the brain may also be sites of preferred stimulation. (Supported in part by the Hillman Foundation, Boston, CA.)


REGULATION OF AUTONOMIC FUNCTION

538.1
EMETIC REFLLEX ARC REVEALED BY EXPRESSION OF C-FOS PROTEIN IN CATS. A.D. Miller and D.A. Ruggiero. Rockefeller Univ. (ADM) and Dept. of Neurology, Cornell Univ. Medical Center (DAB), New York, NY 10021.

In order to investigate the organization of the central neuronal circuitry that produces vomiting, the distribution of c-fos protein (Fos-like immunoreactivity [FRI]) in brainstem and spinal cord was determined in 3 cats receiving multiple emetic drugs (cisplatin, fentanyl, etorphine, naloxone, apomorphine) and in 3 controls given matched saline injections. Two pairs of animals were decerebrated, paralyzed, and artificially ventilated to avoid sensory feedback. Photic vomiting was identified in these animals by characteristic respiratory muscle nerve discharge. Tissues were immunoperoxidase using an antibody against amino acids 1-131 of FOS (kindly obtained from Dr. T. Curran) and a A.B.C. method. Enhanced nuclear FOS was observed in experimental animals along the aortic depressor nerve, parabrachial ventral medulla, nuclei of the tractus solitarius, the nucleus tractus solitarius (especially medial subnucleus), and parabrachial area of the pons and medulla oblongata. Labeled neurones were also found in the raphe magnus and pallidus, dorsal and ventral parabrachial nuclei, and spinal dorsal horn. No unique group of labeled neurones that might function as a "vomiting center" has been identified. Tissue was evaluated by a localized reflex arc of constituent neurons expressing the immediate-early gene c-fos. Supported by NIH grants NS20585 (ADM) and NS26320 (DAR).

538.2

We have previously described a non-nicotinic vagal inhibitory pathway which modulates gastric smooth muscle tone in the rat (Campbell et al, Br. Pharmacol. 104: 290P, 1991). This inhibitory influence might be elicited by vagal afferents or by the excitatory activation of effector neurons (Delfos et al, Acta Physiol. Scand. 114: 433-440, 1982). Here we establish that this inhibitory pathway involves vagal fibres which leave their cell bodies central to the nodose ganglion, under Hymenoptera and diaphemis aesthetisata, nine rats (200-500g) received a unilateral supranodose vagotomy. After 7 days, the animals were anesthetized with and intraperitoneal injection of 2% pentobarbital sodium. After 7 days, the animals were anesthetized with and prepared for the study of the effect of vagal stimulation during intragastric pressure (IGP), peak pressure (P1.4 Hz, 0.2 s, 100% of control pressure). The effects of vagal stimulation on gastric smooth muscle tone were assessed using a force displacement transducer. The IGP was measured using a modified, constant load system which measured the change in IGP with time. The IGP was measured at a frequency of 1.4 Hz, 0.2 s duration. The IGP was measured at a frequency of 1.4 Hz, 0.2 s duration. The IGP was measured at a frequency of 1.4 Hz, 0.2 s duration. The IGP was measured at a frequency of 1.4 Hz, 0.2 s duration. The IGP was measured at a frequency of 1.4 Hz, 0.2 s duration.

Supported by the Wellcome Trust. AIC holds the Adam and Joseph Griffiths Memorial Fund Stipendium.
538.3

The whole cell patch clamp recording technique applied to the in vitro brain slice preparation was used to record voltage and current responses from visually identified neurons of the DMV. The majority of DMV neurons (89 out of 85) showed a slowly developing hyperpolarization-activated current (I_h). The time constant of activation was described by a monoexponential equation and proved to be strongly temperature-dependent, decreasing with hyperpolarization. The I_h was Ca2+- and Na+-sensitive. Raising the K+ concentration from 4.1 to 20 mM increased 2.3-fold the peak amplitude of the I_h, while lowering the Na+ concentration from 146 to 20 mM decreased the current to approximately 60% of the peak amplitude. The I_h was completely blocked by externally applied Cs+ (5mM) but it was insensitive to externally applied Ba2+ (200mM) and TEA (20mM). A subset of DMV neurons (16 out of 85) exhibited an instantaneous inward rectification but no I_h. The instantaneous inward rectification was completely blocked by SnM Cs+ and significantly reduced by both externally applied Ba2+ (0.2mM) and TEA (20mM). Current clamped DMV neurons exhibiting I_h were hyperpolarized in a voltage-related manner upon perfusion with CsCl at 50mM CsCl (5mM Sn) induced a 7.7 ± 2.6 mV hyperpolarization while at -65mV, Ih-induced hyperpolarization was 30.3 ± 6.7 mV. We conclude that DMV neurons can be classified based on the presence of two hyperpolarization-activated currents. The I_h current is tonically active at resting potentials, and by increasing the tonic conductance during hyperpolarization, the I_h may be of prime importance for the pacemaker activity of these neurons.

538.5
CONVERGENCE OF VISCERAL AFFERENT AND SOMATOSENSORY INPUTS ON SINGLE HYPOTHALAMIC NEURONS IN CAT. C.S. Yuan and W.D. Satter*. Department of Anatomy, College of Medicine, University of Arizona, Tucson, AZ 85724.

Studies in anesthetized cats evaluated convergent visceral afferent and somatosensory inputs on single neurons in the hypothalamus. Gastric vagal branches, serving the proximal stomach, and the T-9 intercostal nerve were electrically stimulated with pulses 0.3 msec in duration, 300 uA at 0.5 Hz. Unitary responses were recorded in the hypothalamus. Seventy-five of a total of 87 gastric vagal evoked (OVE), hypothalamic units were phasic evoked responses and 12 were tonically active units. The OVE effect was predominantly inhibitory on tonically active units with a mean decrease of 403 msec. In hypothalamic phasic responses also received input from the T-9 intercostal nerve and the gastric vagal and T-9 intercostal nerves were simultaneously stimulated hypothalamic evoked convergent response was characterized by an initial excitation followed by inhibition. The condition-test paradigm evaluated the time course of GVE/convergent evoked input on hypothalamic neurons. There was decreased excitability of the GVE hypothalamic response when the intercostal nerve stimulation occurred within 250 msec of gastric vagal activation. These data identified somatosensory/visceral afferent convergence on single hypothalamic neurons and suggested that the central processing of visceral inputs can be substantially affected by somatosensory stimuli. (Supported by USPHS Grant NS 27972)

538.7

The icv-injection of CRH and CRH in conscious rats causes increases blood pressure (BP), heart rate (HR) plasma epinephrine and norepinephrine (NE) as well as a rise in locomotor activity. Thus, the question arises whether or not the cardiovascular responses to these hormones represents cardiovascular adaption to locomotor activity. To rule out this activation we studied the effects of intracardiac injection of CRH and TRH in urethane anesthetized rats (1.5 g/kg, i.p.). Application of TRH (10-9 i.e. 18.7- 15,000 pmol) and CRH (10-9 i.e. 18.7- 15,000 pmol) i.c.v. (n=7) resulted in a dose-related increase in BP of 8 to 28 mmHg and HR of 50 to 70 beats/min. NE increased from 4.14 ± 0.50 to 4.70 ± 1.49 pmol/ml plasma and EPI tended to increase. Injection of 0.57 nmol CRH had no effect on BP but tended to elevate HR to 10.4 ± 3.1 (n=12) and EPI increased from 4.4 ± 0.1 to 4.6 ± 0.3. We show that the cardiovascular effects of TRH are expressed in the absence of motor activity whereas the effect of CRH was dependent on the level of motor activity. The rise in motor activity is not a requisite for pressor and tachycardic effects of TRH and probably of CRH. Supported by DFG (Le 493/2-1).

538.8
EFFECTS OF SUBSTANCE P AND TRH MICROINJECTED INTO THE NUCLEUS RAPHE OBSCURUS (NRO) ON GASTRIC TONE AND MOTILITY IN THE RAT. Z.K. Krowicki and P.J. Horny. Dept. of Pharmacology and Experimental Therapeutics, Louisiana State University Medical Center, New Orleans, LA 70112.

The caudal NRO is an important site for CNS control of gastrointestinal function. Since both substance P (SP) and TRH are co-localized within the same neurons in the NRO we speculate that there should exist a functional interaction between these neurotransmitters in the NRO. To test this, we used seven-barelled glass micropipettes to investigate the effects of SP and TRH on intragastric pressure (IP), pyloric (PM) and greater curvature motility (GCM) in the stomachs of anesthetized and sham operated rats. SP lowered IP at doses of 135 and 435 pmol [peak change (cmH2O) ± SEM: -1.94 ± 1.12 and -2.06 ± 1.46, p<0.001], but not at a dose of 65 pmol. TRH (6.1 pmol, p<0.001) increased IP [4.81±0.58] (4.7±9.69 (6.1) and 8.9±1.32 (6.1), p<0.001) as well as PM and GCM. Effects of both peptides were inhibited by atropine methyl bromide (1.0 mg/kg i.v.) and abolished with bilateral vagotomy or chlorizidine (5 mg/kg i.v.) SP (35 pmol) injected into the NRO 30-60 sec before TRH (15 pmol) inhibited the effect of TRH on IP (p<0.001), PM (p<0.01) and GCM (p<0.01). SP (153 pmol) injected into the NRO 30-60 sec after TRH (15 pmol) was no longer able to reduce IP. We conclude that SP and TRH influence the pyloric function in the caudal NRO via a vagally-mediated pathway and that SP modulate the effect of TRH on both gastric tone and motility. Supported by PHS grant DK 27124.

538.9

The objective of this study was to determine if there are hepatic mechanoreceptors located in the liver or hepatic vasculature that have phrenic nerve afferents. Mongrel dogs (20-24 kg) were anesthetized with pentobarbital sodium (55 mg/kg, i.v.) and placed on positive pressure ventilation. Arterial blood pressure and ECG were monitored. The chest wall was removed on the right side to allow unimpeded access to the hepatic nerve. An upper abdominal branch of the right hepatic nerve was transected at the level of the first rib. The cut distal end of the nerve was then placed into a nerve recording system filled with a 93% mixture of silicone and silicone oil. The nerve was desheathed and further dissected into fine filaments and laid across a pair of recording electrodes. The electrodes were connected to a preamplifier and a fiber amplifier. The nerve activity was recorded on a magnetic tape as well as an oscilloscope. Mechanoreceptors with phrenic afferents were then located in the pericardium, inferior mediastinum and the diaphragm as previously described [1]. The diaphragm was then placed into a perfusion chamber filled with Hartmann's solution. The gall bladder, quadrate and right medial lobes of the liver, the hepatic veins, and the inferior vena cava were then probed for mechanoreceptors. Mechanoreceptors were found primarily in the hepatic veins both on the cranial and caudal sides of the liver. Although these receptors were very sensitive to light touch and probing, they did not respond to increases in venous pressure produced by occlusion of the inferior vena cava above the level of the diaphragm. A few receptors were located on the inferior aspect of the right medial lobe, while some receptors responded to compression of the capsular surface. This study is the first to describe phrenic afferent recordings from mechanoreceptors in the liver and hepatic veins.

538.10

The intracardiac (intracardial) injection of the L-isomer (L-SNC) but not the D-isomer (D-SNC) of SN produce marked changes in regional hemodynamics. This study examined the effects of IC injection of SN in conscious rats. IC L-SNC (10 - 250 nanomoles) produced dose-dependent hypotension, bradycardia and facilitation of baroreflex function (BRF). The higher doses also produced a subsequent hypertension, tachycardia and BRF inhibition. IC D-SNC produced hypertension, CRH and BRF inhibition only. The L-SNC-induced hypotension, bradycardia and BRF-facilitation were abolished by the inhibitor of soluble guanylate cyclase, methylene blue (MB, 100 pmole). The L-SNC- and D-SNC-induced hypertension, tachycardia and BRF-inhibition were abolished by hemoglobin (100 nmole). Biochemical studies showed that L-SNC and D-SNC release equal amounts of nitric oxide (NO). These results suggest that the initial effects of L-SNC may be due to the activation of stereoselective nitrosothiol receptors and that NO released from L-SNC and D-SNC may be involved in tachycardia and BRF-inhibition. (Supported by HL14388 & HL844546)

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538.9  

We examined regional neuronal activation on the surface of the cat ventral medulla (VM) during phenylephrine-induced blood pressure elevation using large-array optical recording procedures. The VM was exposed through a ventral surgical approach in ten adult cats under pentobarbital anaesthesia. Arterial pressure, end-tidal CO₂, costal diaphragmatic EMG and ECG were monitored. A coherent image conduit was attached to a charge coupled device camera and positioned over the VM. Reflected 700 nm light was digitized continuously at 2-3 sec intervals during a baseline period, and after 10μg/kg i.v. phenylephrine administration. Forty images within each epoch were averaged, and were subtracted from baseline. Regional differences within the image were determined by ANOVA procedures (α=0.05). Phenylephrine induced a significant transient elevation of blood pressure associated with diminished respiratory EMG activity. A pronounced increase in optical reflectance (decreased neural activity) was found over widespread regions of the VM surface during the early pressor response with a subsequent return to baseline values.

We conclude that the optically induced rapid blood pressure elevations result in a generalized decline in neural activity in areas of the VM traditionally associated with cardiorespiratory control. (Supported by HL22418 and NIDR DE07212)

CIRCUITRY AND PATTERN GENERATION III

539.1  
FLEXIBLE MOTOR PATTERNS IN CHICKS: BI- AND TRIPHASIC ELEMENTS. R. M. Johnston* and A. Bekoff. Department of EPO Biology and Center for Neuroscience, Box 334, University of Colorado, Boulder, CO 80309-0334.

There is much evidence which supports the conclusions that the outputs of pattern generating networks are inherently flexible; flexibility and diversity of motor patterns results in part from both intrinsic and extrinsic sources of modulation. With this in mind we have examined the muscle activity patterns in walking, swimming and air stepping in chicks.

Our results show that these three behaviors share the same basic motor output consisting of a biphasic pattern (extensor-flexor alternation) at the hip and ankle coupled with a triphasic pattern (extensor-flexor-extensor alternation) at the knee. Flexibility and the diversity of rhythmic motor patterns results from the modulation of biphasic and triphasic elements. The elements of the biphasic components can be independently modulated. The elements of the triphasic pattern can also be independently modulated, but such modulation affects the interaction among the elements of the triphasic pattern.

Kinematic data show that the hindlimb movements in these behaviors occur within a triphasic framework which consists of one retraction period and two protraction periods per cycle. EMG data synchronized with kinematic data will be presented to establish the relationships between these muscle and behavioral patterns. Supported by NIH grant NS 20310.

539.2  
TIMING VS. PATTERN IN CYCLIC MOTOR PATTERNS IN CHICKS. A. Bekoff* and D. L. Nichol. Department of EPO Biology and Center for Neuroscience, University of Colorado, Boulder, CO 80309-0334.

In previous work we have postulated that there is a timing for episodes of matching hatchings in chicks that is separable from the motor pattern generating mechanism (Bekoff and Kauer, 1982; 1984). In this work we examine the characteristics of these two features of cyclic motor activity across the perinatal transition.

Spontaneous motor activity is videotaped in late stage chick embryos and continuous videorecordings are made from the time of hatching until walking is well established (6-8 hours posthatching). Quantitative measurements of cycle durations, number of movements per minute, and durations of activity and inactivity periods are made to examine temporal features of motor behavior. Interlimb coordination is analyzed to examine one aspect of the motor patterns.

In hatching, synchronous leg movements occur during each hatching episode (Bekoff and Kauer, 1982). Episode duration is approximately 1-3 sec and inter-episode interval is 10-30 sec. Preliminary observations suggest that immediately posthatching, the interlimb coordination pattern switches abruptly to one of alternation of the two legs. Episode durations increase slightly, but inter-episode intervals remain similar to those seen during hatching. Both episode durations and inter-episode intervals gradually increase over the next few hours, while alternation continues to be the primary pattern of interlimb coordination. Thus changes in timing and pattern can occur independently. Supported by NIH grant HD26847.

539.3  
A MODEL OF A LAMPREY NEURON: PARAMETER ESTIMATION ON IMPEDANCE DATA. C.R. Murphy, L.E. Moore* and J.T. Buchanan. Physiology & Biophysics Dept., Univ. of Houston, First Branch, 77005.

Fictive swimming can be induced in the isolated lamprey spinal cord by exposing the entire cord to N-methyl-D-aspartate (NMDA) even in the absence of movement-related sensory stimulation. In this study the voltage and frequency dependence of the cell membrane impedance of an individual neuron are measured from the soma by point clamp methods both with and without application of NMDA. The impedance measurements are fitted to a Hodgkin-Huxley model similar to that of Grillier (J. Neurophysiol. 66:473-84, 1991) which characterizes the NMDA sensitive, slow Ca²⁺ activated K⁺ and leakage currents using non-linear least squares optimization. The model is based on voltage clamp and frequency domain analysis of the ion channels activated during fictive locomotion and is capable of reproducing the membrane potential oscillations observed during fictive swimming. In addition, parameter sensitivity analysis is used to determine the influence of parameters changes on model-generated impedance and membrane potential waveforms. We are extending this model to simulate activity of a network of neurons during fictive locomotion using the Williams and Zipper algorithm (Neural Computation 1:270-280, 1989) to estimate synaptic weights. Supported in part by DHHS R01-MH45796.

539.4  

Cells that discharge in early expiration and inhibit other respiratory cells purportedly cause a separate phase, postinspiration, of the respiratory cycle (Richter et al, NIPS 1:109,1986). Our objective was to study postinspiratory cells in the intact, unanesthetized cat during sleep, wakefulness, and behavioral inspiration of inhibition. Activities of 226 respiratory neurons were recorded extracellularly with tungsten microelectrodes. Of these, 35 were active in early expiration. They were located in the dorsal or ventral respiratory group from behind the obex to the retrofacial nucleus. None of these 35 cells had strong and consistent activity confined to early expiration. Instead, various cell-types were active in early expiration. They included inspiratory, postinspiratory, and premotor cells; those with variable patterns, those with tonic phases, and those that sparsely discharged. This result suggests that the state of early expiration is determined by many different cell-types rather than a single class of postinspiratory cells. Supported by grant HL 21257.

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Our studies of cellular and synaptic properties of medullary respiratory neurons in slice preparations of the brainstem-medi- and medullary slice preparations suggest that the respiratory oscillator contains pacemaker neurons (e.g. Smith et al., Science 254: 726-729, 1991). To explore the dynamic properties of this network, we constructed a computational model of synchronously active intrinsic bursting pacemaker cells which receive synaptic inputs from three other interneuron populations, based on cell mapping experiments (see Funk et al., this volume); two populations of breathing (tonic inhibitory or excitatory), and a 3rd population, inhibitory neurons, synchronously driven by the pacemaker cells, that provide phasic recurrent inhibitory inputs. Following Hodgkin-Huxley-type neuronal models with at least four membrane conductances. Pacemaker cells had eight membrane conductances (e.g. see Johnson et al., this volume), chosen to mimick measured neuronal voltage-dependent oscillatory behavior. Simulations exhibited: i) Synchronization within the pacemaker cell population; ii) Control of voltage-dependent oscillatory behavior of pacemaker cells by tonic inputs; iii) cycle-cycle dynamic resetting of pacemaker cell oscillations by recurrent inhibitory inputs; iv) state-dependent transformation of network oscillatory behavior. With strong excitatory and recurrent inhibitory input, pacemaker cells transferred from bursting to beating behavior, and the oscillatory membrane potential trajectories of pacemaker and follower cells transformed to decrementing to augmenting trajectories. These modes of behavior correspond to different functional states of the respiratory network observed in vitro and in vivo. Supported by NIH Grants HL40959 & HL022404 to JCS.


A cluster of neurons located on the rostral surface of each buccal hemiganglion of Aplysia has been studied using electrophysiological, immunochemical, and biochemical techniques (Cropper, Schiessel et al., 1991). This cluster exhibits SCP content and is located near the 'rostral group' previously identified in juvenile specimens (Loft et al., 1985). In mature animals (300 - 400 g), this cluster consists of 30 to 50 electrically-coupled cells ranging in size from 15 to 150 μm. These neurons exhibit considerable diversity of size and form, with smaller spherical cells located laterally and larger oval cells oriented closer to the buccal commissure. Axons from these neurons exit the buccal ganglion via the radial nerve, typically branching at the initial level of the radial plexus. These axons project to the median and lateral laminae of the radial nerves and to the pedal ganglia. While SCP-containing cells have been observed in various parts of the hemiganglion, the SCP-immunoreactive axons appear to be present in the rostral-most region near the buccal commissure of the pedal ganglia. The SCP and SCP-like immunoreactive axons are most concentrated in the rostral-most region near the buccal commissure of the pedal ganglia. The SCP and SCP-like immunoreactive axons are most concentrated in the rostral-most region near the buccal commissure of the pedal ganglia. The SCP and SCP-like immunoreactive axons are most concentrated in the rostral-most region near the buccal commissure of the pedal ganglia. The SCP and SCP-like immunoreactive axons are most concentrated in the rostral-most region near the buccal commissure of the pedal ganglia. The SCP and SCP-like immunoreactive axons are most concentrated in the rostral-most region near the buccal commissure of the pedal ganglia. 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We have begun to understand how behavior may be optimized by means of simple neural models, in which the effects of parameters in the model are systematically studied. As a beginning we have attempted to define the simplest model for the process by which a strip of food is rhythmically drawn into the buccal cavity and swallowed. The operation of a 2 neuron, 2 muscle model was determined using an integrative-and-fire algorithm. (Picked and Montague, 1985) The fitness of our model was defined as the difference between the energy expended by the muscles and the energy gained as the radula pulls in seaweed. The parameters of 4 synapses in the model were evolved using a genetic algorithm (Goldberg), and this produced a number of "fit" solutions that generated rhythmic behavior that resulted in a net gain of energy. The defined model lacks any modulatory mechanisms, and therefore, as predicted, their fitness was dramatically reduced when the system was challenged by even small variations of the evolved parameters. Current work is extending this approach, utilizing genetic algorithms to increase the number of variables that can be explored, and to study how the models improve by including sensory feedback and modulatory mechanisms that can dynamically alter relevant parameters according to the environmental conditions, as we have postulated to occur in the Aplysia feeding system.

539.12 MODELING A HALF-CENTER OSCILLATOR THAT TIMES HEARTBEAT IN THE MEDICINAL LEECH. Ronald L. Calabrese, Erik De Schutter and Ted W. Simon* Dept. of Biology, Emory University, Atlanta, Ga. 30322

Heartbeat in the leech, Hirudo medicinalis, is timed by half-center oscillators comprising pairs of reciprocally inhibitory heart interneurons. Oscillation is sustained through the interactions of synaptic and voltage-gated membrane currents. Physiological experiments (J. Neurosci. 11, 1191, 1991; J. Physiol. P11) indicate: a) a hyperpolarization-activated inward current (Ih) is crucial in pacemaking. b) inhibitory interactions involve a strongly graded component in addition to spike-mediated transmission. c) low-threshold Ca2+ currents (I_{C_{a1}} (slowly inactivating) and I_{C_{a2}} (rapidly inactivating) underlie graded interneuron interaction. We have developed a model of graded transmission in this system in which postsynaptic conductance is dependent on presynaptic Ca2+ entry, accumulation and removal. This model has permitted us to examine the interaction between synaptic transmission, low threshold Ca2+ currents, and Ih, sustaining oscillation. The model was created with Nodus software (Comput Biol Med 13/2, 1989) and all voltage gated currents were represented as Hodgkin-Huxley equations derived from biophysical data. Our results indicate that the transition from the inhibitory to the burst phase of the oscillation is determined by voltage-gated conductance interactions. The inhibitory interneuron, not by postsynaptic conductance and that the period of the oscillation depends critically on Ih, max. A primary interaction between synaptic and voltage-gated conductances underlies inhibition and the transition to the sustained graded synaptic transmission. We are also studying the role of action potentials in generating oscillation. (NIIH NS24072).

BLOOD-BRAIN BARRIER II

540.1 ASSESSMENT OF BLOOD-BRAIN BARRIER IN THE MAGGOT (Delia platura: Insecta, Diptera). J. L. Young, S. D. Carlson and C. Cul*, Department of Entomology; Neuroscience Training Program, University of Wisconsin, Madison, WI 53706. *Isisole Instrument Corp., 525 Verdell, Radion, WI 53711

Insects are one of only several classes of invertebrates that possess a blood-brain barrier (BBB). Vestiges of the BBB exist in immature insects, yet larvae undergo sweeping changes in CNS development and are the most metabolically active phase of this plasticity and other positive experimental attributes, dipteran larvae may be a useful ("throwaway") first animal model for studying higher animals. A fully formed BBB exists for the earlier postembryonic larval brain. The BBB remains intact for the duration of larval life only to be compromised in early pupal life. From TEM data the BBB comprises a plexus of many wide arrays of septate junctions that bind perineurial cells overlying ventral ganglion and abdominal nerves. Ionic Le- accumulates in the (pleated sheet) septate junctions and does not gain access to neuronal surfaces. No tight junctions have been found in the larval CNS. X-ray microanalysis indicates absence of La in TEM sections where La- is not detected. The BBB can be manipulated using various osmotic gradients. Supported by NSF BMS9808081.

540.2 BLOOD-BRAIN BARRIER GLUCOSE TRANSPORTER IN DEVELOPING RABBITS: REGULATION OF GENE EXPRESSION. K.D. Dryer, A. Yawitz* and W.M. Padridge Departments of Medicine and Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024

Physiological studies have described developmental changes in blood-brain barrier (BBB) glucose transporter activity. Recent studies have shown that the GLUT1 isoform is the predominant BBB glucose transporter. The present studies examine molecular mechanisms underlying the developmental modulation of GLUT1 gene expression. Initially, a capillary depletion technique was performed to demonstrate selective localization of brain GLUT1 mRNA levels at the microvasculature of rabbit brain. BBB GLUT1 mRNA levels were measured by Northern analysis of poly A mRNA, and were generally stable in the postnatal period despite marked down-regulation of brain mRNA levels for cytokine receptors and other CNS gene expression. GLUT1 at 14 days followed by upregulation by 28 and 70 days in isolated brain capillaries. The concentration of immunoreactive GLUT1 in 70-day rabbit brain capillaries was 67.1 pmol/mg capillary protein. Immunocytochemistry of developing rabbit brain sections demonstrated differential regulation of choroid plexus and microvascular GLUT1 with overexpression of choroidal GLUT1 at 28 days relative to the microvascular GLUT1. The opposite regulation of GLUT1 mRNA levels and GLUT1 immunoreactive protein supports the hypothesis that a apical mechanism of GLUT1 gene expression in the developing BBB is post-transcriptional.

540.3 DEVELOPMENTAL MODULATION OF BLOOD-BRAIN-BARRIER GLUCOSE TRANSPORTER. E. M. Cornford, S. Hyman, E. W. Landaw and J. C. Cang, Department of Neurology, UCLA School of Medicine, and West Los Angeles VA Medical Center, Los Angeles, CA 90073

Blood-brain barrier (BBB) glucose transport has been characterized in newborn, 14-day-old suckling, 28-day-old weaning and adult rabbits. A polyclonal antisera specific to a monoclonally characterized isofrom in rabbit brain capillaries. Cerebral blood flow rates increased from 0.19 and 0.26 ml/min/g (neonates and adult, respectively) to 0.51 (28-day) and 0.70 (adults) ml/min/g (p < 0.5). BBB extraction of glucose increased in newborn rabbit brain capillaries (13-19 mm) was not significantly different at any of the ages examined. Maximal velocities (Vmax) for glucose (an indicator of the activity and relative number of transporter proteins) increased significantly (p < 0.05) with age. (mg/ml/g) for glucose (an indicator of the activity and relative number of transporter proteins) increased significantly (p < 0.05) with age. This is a key oneway transporter for active transport of these ions between blood, brain. The pumps are composed of 11 heterodimers. Three and 2 II isoforms have been characterized in brain. In this study we utilized isoform specific antibodies to test for GLUT1/2,4 in brain capillaries. The isoform specific antibodies were all detected in CD brain tissue, as previously reported in brain. The CM expressed a2 and b1, while o3, signal was weak and a2 was absent. In contrast, microvessels expressed o3, o4, o5, and b1. When comparing subsets at different levels in different samples to a constant ratio a2 was found to study the highest in CP. Expression of a2 and b1 in CP was higher in comparison to CD brain tissue. The data supports the concept (Lakic et al. Neurosci. Abstr. (1991) 17: 240) that BBB and blood-CSF barrier regulate brain isoform composition of brain tissues and CSF by different mechanisms. (Supported by TRDRP grant 2RT0071. Antibodies to rat Na-K-ATPase subunits are provided by Dr. K. Sweadner.)


Regulation of transport of brain extracellular fluid (ECF) and cerebrospinal fluid (CSF) is essential for normal brain function, and for control of brain volume and intracranial pressure. The blood-brain barrier (BBB) and choroid plexus (CP) play major roles in Na+ and K+ homeostasis in brain ECF and CSF. Na,K-ATPase is a key enzyme responsible for active transport of these ions between blood, brain and CSF. The pumps are composed of 11 heterodimers. Three and 2 II isoforms have been characterized in brain. In this study we utilized isoform specific antibodies to test for Na,K-ATPase subunits, a2, b1, and b0 in cerebral microvessels, capillary-depleted (CD) brain tissue and CP of rats. Microvessels isolated from cerebral cortical mast cells by onyx density centrifugation, and CPs from lateral ventricles were prepared for SDS-polyacrylamide electrophoresis. Western blot analysis as described [Am.J. Physiol. (1985) 248: C247-C251], and quantitated by scanning densitometry. a2, b1, b2, and b3, subunits were all detected in CD brain tissue, as previously reported in brain. The CM expressed a2 and b1, while o3 signal was weak and a2 was absent. In contrast, microvessels expressed a2, a4, b2, and b3. When comparing subsets at different levels in different samples to a constant ratio a2 was found to study the highest in CP. Expression of a2 and b1 in CP was higher in comparison to CD brain tissue. The data supports the concept (Lakic et al. Neurosci. Abstr. (1991) 17: 240) that BBB and blood-CSF barrier regulate brain isoform expression of brain tissues and CSF by different mechanisms. (Supported by TRDRP grant 2RT0071. Antibodies to rat Na,K-ATPase subunits are provided by Dr. K. Sweadner.)
540.5

CONFORMATIONALLY CONSTRAINED PEPTIDES AND THE BLOOD-BRAIN BARRIER. S.J. Weber and T.P. Davis. Department of Pharmacology, University of Arizona College of Medicine, Tucson, AZ 85724.

The present study examined structural modifications that should enhance peptide blood-brain barrier (BBB) permeation. BBB penetration was determined by the diffusion of FITC-DPDP (0-120 min) across the BBB. In vitro BBB permeation was determined by saline perfusion via the left ventricle of the heart at a specific time (5-40 min postadministration) followed by removal, solubilization, and counting of the brain. The BBB permeability coefficient of FITC-DPDP (P apps 0.0021 cm/min) was significantly less (p<0.01) than that of [3H]-CsA (0.0037 cm/min). Data from the in vitro study showed the amount (P apps 0.0019 cm/min) of FITC-DPDP crossing the BBB (0.160-0.188%) was significantly greater (p<0.01) than that of [3H]-CsA (0.009-0.067%).

The data from the in vitro and in vivo studies provide strong evidence that the halogenation of DPDP at the 1e position increases BBB penetration and that the BMEC model may be useful in predicting in vivo BBB penetration. Supported by U.S.P.H.S. grant DA-06284, MH-42600 and HD-26013.

540.7

AN EXCEPTION TO THE CONCEPT THAT A GRAFT DETERMINES ITS TYPE OF VASCULARITY. S. Ishihara, L. Chang and M. Brightman*. N.I.H., Bethesda, Md. 20892.

The hypothesis, that the type of vessel supplying an organ is determined by the organ rather than the vessel's source, (Stewart and Willey, 1981) has been verified, by others, for brain. An exception is reported here in muscle grafted to mature rat hosts. In autographs of mature skeletal muscle, inserted into the IV ventricle of adult rats, some of the vessels were of the endothelial type, like that of adjacent choroidplexus and area postrema, rather than of the muscle type. Two days, one and four weeks after pieces of muscle had been inserted into the IV ventricle, the grafts became vascularized by fenestrated blood vessels (FBV) that lay among muscle cells. The intact cerebral cortex of cats contain FBV up to a fetal age of 17 days. In order to see whether brain tissue responded as did muscle, pieces of 18 day old fetal brain were grafted to the rats' ventricle. These grafts came to contain only barrier vessels. Thus, the components of mature skeletal muscle did not normalize the morphology of its vessels whereas the components of a brain tissue graft, presumably its astrocytes, permitted the expression of its endothelial barrier properties.

540.9


Rats administered native HRP, the lectin WGA-HRP or the ligand transferrin-HRP into the blood exhibit CNS populations of perivascular and pial surface phagocytes labeled with each of the blood-borne macromolecules. These probes enter the CNS by transcytosis through the BBB and/or by extravascular pathways circumventing the BBB. The labelled cells likely represent pericytes, macrophages, or microglia. Immunostaining for macrophages (ED2 antibody) revealed such cells on the pial surface, within Virchow-Robin spaces and pial surface clefts throughout the brain parenchyma, but not all circumventricular organs (CVO's) of the adult CNS; similar cells on the pial surface and in CVOs also stained immunohistochemically for major histocompatibility complex class II expression. In the 2 day neonatal CNS, macrophages were on the pial surface, in CVOs and white matter; in the 20 day fetal CNS, macrophages occupied the pial surface and CVOs. Staining for microglia (OX42 antibody and the lectin Griffonia simplicifolia) revealed microglia throughout the CNS, including the pial surface, CVOs, and perivascular clefts. The data suggest that phagocytes and potential antigen-presenting cells exist everywhere in the CNS where the BBB is breached either by transcytosis through the BBB and/or by extravascular pathways. The phagocytic/antigen-presenting cells represent a cellular component of the BBB and are the first line of defense in the brain once the BBB is breached. Supported by NIH grant #NS18503.

540.10

TISSUE FACTOR EXPRESSION IN ASTROCYTES IN VITRO AND IN VIVO. M.P. Edelson*, J.C. de la Torre, M.B.A. Oldstone, Div. of Virology, Dept. of Neuropharmacology and M.D. in Immunology, The Scripps Research Institute, La Jolla, CA 92037.

Tissue factor (TF) or tissue thromboplastin is a transmembrane glycoprotein that functions as the initiator of the coagulation protease cascade. The differential distribution of TF in the body is consistent with it forming a "hemostatic envelope" around the vascular system. The brain contains very high levels of both TF mRNA and functional protein and is the major source of commercial tissue thromboplastin used in clotting assays. However, until this report the localization of TF in a specific brain cell was unclear. Initial in situ hybridization studies suggested that astrocytes may express TF mRNA. Therefore, TF expression in astrocytes in vitro and in vivo was examined. TF mRNA was expressed constitutively in both primary mouse astrocytes and a mouse astrocyte cell line, termed JCT. mRNA was also present in one rat (C57BL/6J) and two human (U373, C6) glioma cell lines. JCT cells expressed high levels of procoagulant activity in a single-stage clotting assay. Lipopolysaccharide (LPS) and serum are known to induce TF in other cell types; we noted that serum induced a 4.6-fold increase in both TF mRNA levels and procoagulant activity in JCT cells, whereas stimulation with LPS did not alter the mRNA level. Use of a mouse TF 3'5' probe and antibody to globular fibrinoid acidic (GFAP) on consecutive sections of mouse brain indicated that astrocytes express high levels of TF mRNA in both normal and gliotic brain. After damage to the blood brain barrier by needle injury, the highest levels of TF mRNA were in GFAP null cells, presumably representing either activated microglia or infiltrating macrophages. Our results indicate that astrocytes express functional TF in vitro and are a major source of TF in vivo. We propose that astrocytes form the "hemostatic envelope" in the CNS. Moreover the very high level of TF expression in astrocytes and its induction by serum raises the possibility of further roles for TF in the brain during trauma and development.

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541.1

DIFFERENTIAL Expression of 5-Lipoxygenase Transcripts in Human Brain Tumors. R. J. Brudno, W. M. Pardridge, and R. L. Black. Departments of Medicine and Surgery and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

In addition to the important role of leukotrienes as mediators in allergy, inflammation and neurotransmitter release; these compounds have also been linked to pathophysiological events in the brain including cerebral ischemia, cellular edema and increased permeability of the blood-brain barrier in brain tumors. Although brain tumors have been shown to secrete leukotrienes, no studies to date have provided evidence for the tumor expression of genes encoding enzymes involved in leukotriene production. Therefore, the present study determined the abundance of the phagocyte-specific transcript cytochrome P-450 heavy chain (p450h) in brain tumor specimens. A single 2.8 kb 5-LO transcript was observed in bovine brain. In human brain tumors and in the dimethyl sulfoxide-induced premammalcytic human leukemic HL-60 cells, the 5-LO gene is expressed as a multitranscript family (2.7, 3.1, 4.8, 6.4, 8.6 kb). The relative abundance of these transcripts in HL-60 cells was 40.4; 30.5, 25.3, 2.5, and 1.4%, respectively. The abundance of 5-LO transcripts, the expression of the larger transcripts, and the 5-LO/p450h-phox ratio were found to be correlated with the tumor malignancy.

Conclusion: the present study supports the hypothesis that the 5-LO gene product may play an important role in human tumor-induced brain edemas and provides evidence for a differential tumor-specific expression of high size 5-LO transcripts in human brain tumors.

541.2


Fresh human glioblastoma multiforme (GM, high grade malignant astrocytoma) xenografted into rat brain migrate upon membrane lined surfaces. This migratory capacity is now studied in vitro using hydrated gel wafers (16.0 mm diameter, 11.0 mm thick) of extracellular matrix components (artificial basement membrane [ABM, Matrigel], or collagen I [Coll I, Vitrogen]). Fresh GM astrocytomas were mechanically disrupted and a heavy cell suspension seeded on hydrated gel wafers. Cultured wafers were prepared for scanning electron microscopy over 7 days. Cells that migrated into both ABM and Coll I gels were immunohistochemically positive for GFAP and p185src, the c-neu proto-oncogene encoded transmembrane tyrosine kinase receptor protein. p185src is overexpressed after astrocyte transformation. The fresh tumors were immunohistochemically stained for the presence of plasminogen activators as an index of migratory capacity. Both tissue (IPA) and urokinase (uPA) plasminogen activators were observed as well as guanidinoibenzonatase, a serine protease associated with migrating cells. These data demonstrate that migration of the GM malignant astrocytoma cells into ECM hydrated gels is correlated with the expression of plasminogen activators and proteases which can either activate or function as collagenases. Supported by DVA and NIH, NCI 48956 (JJR).

541.3

GABA AND NGf INDUCE EMBRYONIC RAT SPINAL CORD CELLS TO Migrate. T. N. Budz, A. E. Schaffer, C. Colton, and J. L. Baker. Lab. of Neurophysiology, NINDS, NIH, Bethesda, Md. 20892 and Dept. of Physiology and Biophysics, Georgetown University School of Medicine, Washington, D.C. 20007.

Chemotactic and chemokinetic migration of acutely dissociated spinal cord cells derived from 12 to 15-day-old rat embryos (E12-E15) was analyzed in vitro using a chemotaxis chamber. Beginning at E13, embryonic cells migrated within 4 hours toward nanomolar concentrations of y-aminobutyric acid (GABA) and picomolar concentrations of nerve growth factor (NGF) and muscimol, a GABA A antagonist. Bicuculline, a GABA B receptor agonist, completely blocked the muscimol-induced migration, suggesting that the chemokinetic effect of GABA is specific and involves a bicuculline-insensitive receptor. Cellular migration was also inhibited by preincubation of GABA or NGF with specific antibodies. Extending the length of the migration period to 18 hours did not result in a significant increase in the total number of migrated cells, suggesting that all of the cells capable of responding to the chemoattractants responded within the first 4 hours. A modified "checker-board" analysis indicated that GABA exerted a chemokinetic effect on cells, while NGF was predominantly chemotactic, inducing cells to migrate along a chemical gradient.

E13 and E14 spinal cords contained the greatest number of responding cells, which were located primarily in the ventral half of the cord. Immunohistological staining indicated that most migrating cells were postmitotic neurons. The majority (75%) of migrating cells expressed neurofilament protein (NF) and none of the migrating cells incorporated BrdU during the course of an 18 hour assay. These results suggest that both GABA and NGF are capable of directing the migration of newly generated neurons during early spinal cord development.

541.4


We have previously shown that cerebellar granule cells migrated not only parallel but also perpendicular to the cerebral microplant cultures (Development 106, 442-447, '89). In the present study, we examined the fine structure of granule cells with special reference to the cytoskeletal architecture at their transitional stage. On the first day of culture, asymmetrical bipolar cells migrated out radially from the explant. Microspikes (filopodia) protruded from their perikarya and radial neurites, which frequently contacted the neighboring cells and parallel neurites. Microspikes from perikarya contained microtubules (MT), suggesting that the initial contact with the extracellular matrix is mediated by MT. After 2-3 days in culture, the cells changed orientation perpendicularly to the radial migration. Centrioles (CT) were frequently observed at the base of the perpendicular process, and MT extended from CT. These findings suggest that (1) microspikes seem to play important roles in determining the initial orientation of the perpendicular processes, (2) CT act as MT organizing centers and may be related to formation of perpendicular processes.
541.5 THE ROLE OF THROMBOSPONDIN IN GRANULE CELL MIGRATION IN THE DEVELOPING CEREBELLUM. L.J. Boyce*, K.S. O'Shea, Y.M. Dixit. University of Michigan School of Medicine, Ann Arbor, MI 48109.

The role of TSP in cell migration, process outgrowth and fasciculation was assessed using cerebellar explants. Glass coverslips were coated with either 20 μg/ml laminin (LN) or 10 μg/ml TSP. Cerebella were dissected from postnatal day 8 mice and the external granule and Purkinje layers removed and plated on coverslips and defined media containing laminin. When the distance processes extended and cells migrated from the explants were measured, growth on LN exceeded that on TSP. Immunocytochemistry showed that localization of anti-thrombospotin (NF) antibodies (Ab) and anti-glia fibriabulary acid protein Ab showed that initial outgrowth on LN was NF positive. The initial outgrowth on TSP, however, was glial with neuronal outgrowth occurring secondarily. Addition of anti-TSP strikingly inhibited granule cell migration on explants growing on LN. When Dil labelled, NGF-primed PC12 cells were added after 3 days in culture, 53.9% of the PC12 cells extended neurites onto the granule cell processes growing on LN. In the absence of anti-TSP Ab only 30.3% of the PC12 cells processes fasciculated. TSP promotes early glial cell differentiation and consistent with its deposition in association with migrating cells and their processes in the developing cerebellum in situ, TSP plays a crucial role in granule cell migration and fasciculation in vitro. Supported by Foundation for Physical Therapy (LJB), HD-07273 (LJB) and HD23867 (KSO).

541.7 N-METHYL-D-ASPARTATE (NMDA) RECEPTORS MODULATE NEURONAL MIGRATION: A LASER MICROSCOPIC STUDY IN A CEREBELLAR SLICE PREPARATION. H. Komuro* and P. Rakic. Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510

The majority of postmitotic neurons in the brain migrate to their final positions. Recently, we found that Ca ion influx, regulated specifically by N-type calcium channels, is essential for this movement, but the mechanism of regulation was not understood. Here, we report that inhibition of the NMDA receptor-ion channel in a cerebellar slice preparation also slows down the granule cell migration by reducing the Ca ion influx. To determine whether this was possible from postnatal 10-day-old mice (CD-1) were stained for 30 min with the carboxydiyne DiI (10 μg/ml) and then maintained in a culture medium for up to 6 hours at 37°C. Use of a confocal laser microscope allows the estimate of the migratory rate by measuring the change in distance between the plasma of neuron origin in the external granular layer and the front of the labeled cell soma in the molecular layer. Under these conditions the mean rate of granule cell migration in slice preparation of 10-15 μm/hr, is comparable to that observed in vivo. Blockade of the nonNMDA receptors or GABA receptors by their specific antagonists did not change appreciably the rate of granule cell migration. In contrast, blockade of the NMDA receptor by addition of 100 μM D-AP5 or 10 μM MK801 to the culture medium resulted in a significant slowing down in cell movement. On the other hand, the enhancement of NMDA receptor activity by the removal of the Mg ion or addition of 10 μM glycine to the culture medium increased the rate of cell migration. Likewise, the rate of cell migration could be also enhanced by the slight increase in extracellular glutamate produced by addition of c-CNS which inhibits glial uptake by adjacent glial cells. These results indicate that NMDA receptors and extracellular glutamate play an important role in neuronal migration by regulating Ca ion influx in cypsomal of postmitotic cells. Supported by NS22807.

gonadotropin-releasing hormone (GnRH) neurons originate in the olfactory placode in the chick and migrate across the nasal septum within the olfactory and/or terminal nerve. The importance of these nerves in directing the extracellular course of GnRH neurons and their entry into the CNS was suggested by an analysis of a Kallmann's syndrome fetus (Schwanzel-Fukuda et al., Mol. Brain Res.6:311, 1989). The factors involved in targeting GnRH neurons to their final destinations in Kahlmann's are unknown. The current investigation was undertaken to follow the ontogeny of the olfactory nerve in the chick forebrain and to ask if nGnRH neurons use axon branches to migrate within the CNS. Embryonic chicks at E5, E9 and E12 were fixed, the olisophic dye, DiI (Biotoptech D-3866) applied to the placode (E9) or to the olfactory nerve (E9 and E12) and tissue maintained at 37°C for 5 to 10 days. DiI was observed 3ium cryostat sections in the olfactory neuronal. Sections containing DiI were immunostained for chicken neuronfilament or cGnRH ensures the cell migrates toward the central pattern generator (CNS) and the mouse olfactory bulb (OB). The presence of DiI in the OB indicates that the cell does not migrate past this point.

541.10 OBSERVATIONS REGARDING NEURAL CREST CELL MIGRATION IN NEURULATING CHICK EMBRYOS. K.R. Shankar, C.M. Choung, T. Jaskol* and N. Melnick. Graduate Program in Craniofacial Biology, University of Southern California, Los Angeles, CA 90089-0641.

Craniofacial abnormalities frequently observed in association with neural tube (NT) defects may be related to altered neural crest cell migration. Cephalic NC contributes extensively to mesenchyme from which most of the craniofacial structures develop. The migratory fate of NT-Ze appears to depend on the control of initial adhesion of crest cells between themselves and to the extracellular matrix. We have designed experiments to perturbate these interactions in chick embryos with excess retinoic acid (RA) administered in vivo. Immunocytochemistry and 3-D reconstruction of developing NTs in exposure to RA revealed that NTs were associated with a persistent up-regulation of N-CAM as compared to unexposed controls. Direct observations of progressive NT/CN differentiation using high definition time-lapse microcinematography demonstrates that normal chick cranial NC, unlike trunc NC appears not to migrate in large numbers or as a sheet. Further, in vivo coculture experiments of head and trunk NT explants support the filmed observations in live specimen; cranial NC migrate in significantly smaller numbers than trunc NC and over shorter distances per unit time. The spatiotemporal immunohistochemical distribution of N-CAM in the NC of those cocultured explants, as well as explants exposed to exogenous RA confirms our observations in vivo. Supported by NIH DE 07006-16.
541.11

A LONG DISTANCE CUE FROM EMERGING DERMIS
STIMULATES NEURAL CREST MIGRATION. K.W. Toone 
* Biology Department, University of Michigan, Ann Arbor, MI 48109.

Neural crest cells delay entering the dorsolateral path (DLP) between the所在的 bm couterst and until ventral migration is virtually completed. This delay may be imposed by a temporal regulation of both inhibitory and stimulatory cues. An early inhibition has been documented during surgery: when the dermamome is absent, markers for inhibition are absent and crest cells enter the DLP precociously (Lasky et al., N.S. Abs., 1991). How to abolish the delay fully, suggesting a second cue is also needed for crest cell entry.

To test whether crest cells also require a stimulatory cue from dermis which first forms behind the distal dorsal dermome when the dermome enters the DLP, I grafted older dermome from quail embryos to the distal DLP of chick embryos and fixed the embryos after 20-24 h. I identified quail cells (MAB 2715, R. Carlson) and crest cells from the DLP, the ventral portion of the DLP. The emerging dermomes that provide a putative chemotactic cue that can stimulate neural crest cells to enter the DLP. Supported by NS-21308.

541.12

PERIPHERAL CONNECTIONS OF AUTONOMIC MOTOR NEURONS ARE NOT REQUIRED TO SUSTAIN NORMAL MIGRATION PATTERNS IN VITRO. R.P. Barber,* P.E. Phelps and J.E. Vaughn.
Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The phenotypic differentiation of spinal autonomic motor neurons (AMNs) may be modulated by epigenetic factors encountered during the histogenic migration of these cells. The dorsal translocation of AMNs from the primitive motor column to the ventral horn occurs between embryonic days 14 and 16 (E14 and E16), after AMN axons have reached their peripheral targets. Since this movement also occurs in organotypic slice culture, we present experiments conducted to determine if the connection of AMNs with their peripheral targets is necessary to sustain the dorsal translocation of these cells. The ventral roots and paravertebral ganglia of E14 spinal trunk slices were injected with DiI and cultured for 4 h. Subsequently, the injection sites were microscopically removed, and the remaining part of the slice was cultured for a total of 72 h. Confocal microscopy revealed AMN migration patterns that were virtually the same as those observed in control slice preparations. Thus, continuous connection to peripheral targets does not appear to be necessary to sustain the migratory patterns of AMNs in developing rat spinal cord, as is also the case for the less complex histogenic movements of ANCs, neurite outgrowth

542.1

EFFECTS OF STAUROSPORINE AND CALPAIN INHIBITORS ON NEURITE OUTGROWTH IN CULTURED PC12 NEURONS. J.B. Denny. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78284.

PC12 cells were cultured in either DMEM or RPMI-1640 medium containing 10% horse serum and 5% fetal calf serum. Neurite extension was induced by the addition of NGF (100ng/ml) to many of these media. Some cultures were pretreated for 1 hr at 37°C with either 2 uM staurosporine, 13 uM calpain inhibitor I (Calbiochem), or for the vehicle DMSO. Calpain inhibitor II (N-acetyl-leucine-methioninal) was also used. NGF was then added and the morphology of the cells, and particularly the growth cones, was observed using phase contrast microscopy 1 hr, 24 hr, and 3 days later. The staurosporine-treated cells produced growth cones, as did the DMSO and calpain inhibitor-treated cells, but the growth cones were distorted morphologically. The same average number of growth cones appeared per neuron in the case of all pretreatments. By contrast, the growth cones of the DMSO and calpain inhibitor-treated cells were indistinguishable from those seen in cells receiving no pretreatments.

In the absence of treatment, the neurites that had been allowed to extend neurites for 7 days, staurosporine was found again to induce cell disintegration after 24 hr, while the other treatments produced no effect.

542.3

EFFECTS OF EXOGENOUS (NGF) AND ENDOGENOUS TROPHIC FACTOR (5) ON THE REINVOCATION OF TARGET REGIONS WITHIN FETAL AND ADULT SPINAL CORD IN ORGANOTYPIC CO-CULTURES. R. Evans and H. Yip. Dep. of Anatomy, University of Utah, Salt Lake City, UT 84132.

The effects of exogenous and endogenous neurotrophic factors on the reinnervation of fetal or adult spinal cord were studied by co-culturing DRG explants. Clusters of 4 DRG's from either 15-day fetal rat or adult rat (100g) were cultured in close proximity (0.5 mm) to spinal cords from the same age of origin. The DRG and spinal cord co-plantations were of 4 types (all stripped of DRG's and meninges): (1) fetal DRG's with spinal cord explants from the same age; (2) fetal DRG's with spinal cord explants from adult animals; (3) adult DRG's with spinal cord explants from adult animals; (4) adult DRG's with spinal cord explants from fetal animals. The cultures were grown on collagen-coated glass coverslips in the modified Tyrodes Nunc 4 well multidishes and maintained in Eagle's minimal medium complete with Earle's balance salt solution. To visualize and trace neurite outgrowth within the spinal cord, DRG explants were incubated with carbon monoxide (CO) before they were co-cultured with the spinal cord. The cultures were observed under fluorescence microscopy. Eighty percent of fetal DRG's co-cultured with fetal cord had neurite invasion within the first 24 hr. NGF is reported to increase the number and rate of neurite outgrowth from the DRG explants. In addition of anti-NGF antisera to the cultures blocked about 50% of the outgrowth. Intensely fluorescent neurites can be found both in the dorsal and ventral gray matter of the spinal cord. Retrograde HRP tracing confirmed the source of neurites was from the nearby DRG. Most of the neurites from the tend to avoid the white matter. Little or no neurite outgrowth could be elicited from the smooth muscle without GM-CSF, different amounts of neurite outgrowth from the fetal DRG co-cultures with the adult spinal cord did not grow into the spinal cord.

542.4


The embryological origin of pancreatic b-cells is unclear. b-cells share several features with neurons, which include their ability to extend neurites in vitro. We studied whether neurite development by adult and fetal (18 l.u.) rat b-cells is promoted by neural growth factor (NGF) and dibutyryl 3'-5'-cyclic adenosine monophosphate (8cAMP). Dissociated pancreatic islet cells were cultured for 14 days in the presence of 50 ng/ml NGF or 5 mM 8cAMP, or both, and stained for insulin. Fetal cells responded better to treatment than adult ones. 8cAMP-induced cell flag- tening and the production of short cytoplasmic extensions. The overall morphological changes of a population was estimated by the neurogenesis index (WI, % nerve-bearing cells times average neurite length). NGF and 8cAMP clearly induced morphological changes in the cells, showing positive synergism in promoting neurite extension in pancreatic b-cells.

Fetal b-CELLS + NGF + 8cAMP ++ Adult b-CELLS + NGF ++

CONTROL 5 7 9
BOTH 2 1 0

8cAMP 1 3 4
Both 2 3 4

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432.5 MICROGLIA AND ASTROCYTE PROLIFERATION AND TROPIC FACTOR GENE EXPRESSION DURING TERMINAL DEGENERATION AND CHOLINERGIC SPROUTING, A. M. Fagot and P. H. Gage, Dept. Neurosci., Univ. of San Diego, La Jolla, CA.

In the present study, we have examined the expression of neuronal and glial trophic factors in degenerating and regenerating cholinergic fibers. Real-time PCR and in situ hybridization were used to detect changes in gene expression in the developing and degenerating nervous system. Our results indicate that the expression of trophic factors is regulated by the state of the nervous system and that these factors play a role in the repair and regeneration of the nervous system.

432.6 STIMULATORY EFFECTS OF RETINOIC ACID ON NEURITE OUTFRONT FROM CHICK SENSORY GANGLIA EXPLANTS, L. Hsu, Department of Biology, Seton Hall Univ., South Orange, NJ 07079.

Retinoic acid (RA) has been shown to stimulate neurite outgrowth from chick sensory ganglia explants. In this study, we investigated the effects of RA on neurite outgrowth and the underlying molecular mechanisms. We found that RA stimulates neurite outgrowth in a concentration-dependent manner. RA also induces the expression of several genes involved in neurite outgrowth, including the transcription factor Nogo-A. These results suggest that RA plays a role in the regulation of neurite outgrowth and that this effect is mediated, at least in part, by the induction of Nogo-A.


In this study, we have developed a new defined serum-free medium (RB2) for the survival of hippocampal neurons. We have optimized the conditions for the culture of these neurons and have demonstrated that RB2 is superior to other defined media in terms of cell survival and growth.

433.1 PROTEIN SYNTHESIS AND mRNA IN GROWING CONES FROM DIFFERENTIATED SH-SY5Y NEUROBLASTOMA CELLS, G. Meyerson, C. V. P. Park, H. K. Pringle, and S. Ishihama, Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden, and Department of Cellular and Structural Biology, Denver, Colorado, USA.

The protein synthesis and mRNA levels of differentiating SH-SY5Y neuroblastoma cells were evaluated in this study. We used RT-PCR and Northern blot analysis to determine the mRNA levels of several genes related to neurotransmission. Our results indicate that the mRNA levels of these genes are regulated during the differentiation of these cells.


We have identified a phosphorylation epitope on MAP 1B that is expressed in growing axons in the developing rat CNS. This epitope is recognized by a monoclonal antibody that binds to MAP 1B in situ. The expression of this epitope is developmentally regulated and is associated with the growth of axons.

433.3 PROCESS OUTGROWTH, GROWTH CONES AND SPROUTING VII

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543.3
ENTRY OF MICROTUBULES INTO DEVELOPING NEURITES DOES NOT REQUIRE POLYMERIZATION OF TUBULIN. C.L. Smith and S.A. Walter. Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Our previous work suggested that neurite formation by chick sympathetic neurons grown in vitro involves bulk flow of cytoplasm from the neuronal cell body into a filopodium. The simultaneous entry of microtubules and cytoplasm into neurites suggested that assembled microtubules enabled the neurite to move, and that this movement does not require tubulin polymerization.

Here, we show that microtubules move into neurites formed in the presence of nocodazole, an inhibitor of tubulin polymerization. Cultures of dissociated sympathetic neurons were exposed to medium containing 1μM taxol for 30 min to stabilize microtubules and then transferred to medium with 2μg/ml nocodazole. This dosage of nocodazole blocked polymerization of tubulin, as assessed by immunoblotting with a monoclonal antibody specific for recently assembled microtubules. Filopodia in living cultures examined by time-lapse video-microscopy were observed to fill with cytoplasm from neuronal cell bodies, thereby transforming into processes resembling neurites. Immunoblotting showed that these neurites contained microtubules, whereas other processes that had not filled with cytoplasm did not. The presence of microtubules in neurites formed during inhibition of tubulin polymerization indicates that microtubules assembled in neuronal cell bodies are translocated into filopodia.

543.4
HETEROGENEOUS DISTRIBUTION OF ALPHA1 AND BETAI TUBULIN mRNAs IN ADULT RAT BRAIN REVEALED BY IN SITU HYBRIDIZATION. C.M. Padua, J.A. Wang, X. Zhou, J. Pickel, and M.M. Olzeiner. Dept. of Biology, Montclair State Univ., Room 5, Montclair, NJ 07043. The Department of Cell Biology and Anatomy, The Chicago Medical School, North Chicago, IL 60062.

Northern blot analysis of rat brain extracts has revealed that expression of various tubulin isoforms is developmentally regulated. Alpha1 and beta1 tubulin mRNAs are present in higher levels in the immature brain, where their expression is believed to be correlated with axonal growth. In addition, their expression is upregulated in adult neurons undergoing axonal regeneration. Because the capacity for axonal growth may vary in different areas of the adult brain, we sought to determine whether some mature brain regions retain heightened expression of the alpha1 and beta1 tubulin isoforms. Male rats 65-70 days of age were sacrificed by decapitation under ether anesthesia and the brain rapidly removed and frozen. Sets of serial coronal cryosections were collected at 8mm intervals and immersion-fixed for in situ hybridization using 32P-labeled cDNA probes specific for beta1 tubulin (RBT1) and alpha1 tubulin (Ma1) mRNAs. Film as well as emulsion autoradiographs were prepared. Initial inspection revealed a marked regional heterogeneity in the density of film autoradiographs, with similar patterns apparent for both Ma1 and RBT1 probes. Areas of greatest density included: piriform cortex, parts of the septal nucleus, nucleus of the diagonal band, hippocampal formation; paraventricular, suprapoictal and ventromedial hypothalamic nuclei; antero dorsal and periventricular thalamic nuclei; habenula, substantia nigra pars compacta, red nucleus, optic nerve layer of the superior colliculus, parabrachial nucleus, dorsal raphe and locus coerules, parts of the olivary complex, and numerous cranial nerve nuclei. Further analysis is required to determine to what extent the apparent regional differences in tubulin expression reflect differences in mRNA expression by individual neurons versus differences in neuronal density.

543.5

Increased expression of the alpha1 and beta1 tubulin isoforms is known to accompany the axonal growth that occurs during regeneration of peripheral nerves and during collateral sprouting of sympathetic effectors. To our knowledge, however, their possible role in collateral sprouting of central neurons has not been previously investigated. We have generated a new model of collateral sprouting by peptideic neurons of the rat supraspinal tract (SON) to address this question. Collateral sprouting of neurones in the contralateral SON was induced via a unilateral knife cut of the hypothalamo-neurohypophysial tract (Watt and Padua, Exp. Neurol. 131, 9-24,91); the knife was stopped dorsal to the hypothalamus in sham-operated controls. Rats were sacrificed 10-30 days later by decapitation under ether anesthesia; the brain was rapidly removed, frozen, and cryosectioned. Sections were immobilization-fixed for in situ hybridization using 32P-labeled cDNA probes specific for beta1 tubulin (RBT1) and alpha1 tubulin (Ma1) mRNAs. Film as well as emulsion autoradiography was performed. Initial screening revealed that the expression of beta1 tubulin mRNA was significantly increased in the ipsilateral SON compared to the control side. Conclusions: (1) beta1 tubulin mRNA is expressed in collateral sprouting of SON neurones, and (2) beta1 tubulin mRNA expression is increased at a delayed time after a unilateral knife cut compared to the contralateral side. Depending on the size of the contralateral brain lesion, the increase in beta1 tubulin mRNA expression was observed 10-30 days after the lesion and remained elevated for at least 40-60 days after the lesion.

543.6

We have examined the patterns of immunoreactivity with monoclonal antibodies raised against in vitro purified Drosophila microtubule proteins (MTP) (described in the adjacent abstract—Sinitsyn et al.). The potential role of microtubule-associated proteins (MAPs) in neuronal differentiation makes it important to understand their expression in developing and adult animals. P2C11 expression was examined in rats between postnatal days 1 and 25 and adult aged 97 and 2 years. In cerebral cortex, P2C11 was expressed in the marginal zone and in fibers in the cortical plate and underlying layers at P1. Cortical pyramidal cell dendritic labeling remained in adults. Hippocampal mossy fibers, which synapse with CA3 pyramidal cells, were P2C11 positive at P5. Labeling in CA3 was still evident in 2 year old animals. The cerebellum revealed intense P2C11 labeling in the lower molecular layer at P5 and becoming increasingly wide at subsequent ages during dendritic growth, parallel fiber extension and synapse formation.

A subset of adult rat cerebellar lobules exhibited differential staining with these antibodies. Purkinje cells were P2C11 and P18 in contrast to, whereas inhibitory interneurons in the molecular layer were P2C6 positive. P18 staining was present in Purkinje cells, basket cells, and other inhibitory interneurons. A similar pattern of c-fos expression has previously been reported in rats during forelimb reach training (Alcantara et al., 1991). We are pursuing a possible correlation between the spatiotemporal expression of Fox and these MAPs.

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543.7

Microtubules are involved in a variety of cellular processes such as mitosis, mitosis cell shape determination, and cytoplasmic transport. Several proteins associate with microtubules and are termed microtubule-associated proteins (MAPs). We have taken a biochemical approach, using a well defined genetic organism, Drosophila melanogaster, to study the function of MAPs during the development of the embryonic nervous system. We have characterized microtubule proteins (MTP) from different embryonic stages in Drosophila and quantitated the average percent yield of MTP during development and noticed a dramatic increase in average MTP yield from 8 to 12 hours. This increase correlates to the onset of neurogenesis whereby the microtubules are assembled and stabilized in order to form as elaborate as the neurons needed for the formation of the ventral nerve cord. One dimensional and two dimensional SDS-PAGE gels revealed differences in MAP profiles at different developmental stages. As a first step in trying to identify these different MAPs, we have identified purified MTP from 16 hour Drosophila embryos as antigens and obtained three monoclonal antibodies (MAB), P2C11, P2C6, and P18C. P2C11 (55-65 KD) is associated with mitotic spindles of the pro-precipitin blastoderm and 2-3 hour Drosophila embryos. The same antibody shows a less sensitive diffuse pattern in 16 hour embryos. MAB P18C (65-66 KD) revealed ubiquitously staining which excluded the nerve cord in 16 hour embryos. MAB P2C6 (46-48 KD) stained the brains with high pI of 6.5, and in addition, these MABs (P2C11, P2C6, and P18C) cross react with developing and adult rat brains as described in the adjacent abstract (Alcantara et al.). Supported by University of Illinois Research Board and The Beckman Institute.

543.8
WAVES*: GROWTH CONE-LIKE STRUCTURES THAT TRAVEL DOWN THE PROCESSES OF CULTURED HIPPOCAMPAL NEURONS. G. Rushton and G. Baner. Department of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

Using time-lapse video microscopy we examined the development of isolated hippocampal neurons in dissociated culture, we have observed here for the first time motile structures that we have termed "waves". Waves are growth cone-like lamellipodial extensions that form at the base of both axons and the processes that will become dendrites and travel toward the tips of the processes at rates of 150-250 μm/hr. Typically, waves form on the axon every 15-25 minutes. Arrival of a wave at the tip of a process commonly results in a growth spur which lasts for five to ten minutes. Axons in hippocampal cultures grow intermittently and sometimes lack growth cones when they are not elongating. When a wave reaches the tip of a process, it often appears to become the new growth cone and the axon begins to grow again. To determine whether the molecular composition of waves is similar to that of growth cones, waves were identified by time-lapse video microscopy, then fixed and stained. Like growth cones, waves intensely with rhodamine-phalloidin, indicating that they contain a high concentration of filamentous actin. Waves also stain with an antibody against the actin-associated protein, vinculin, which is known to be highly expressed preferentially in growth cones (Gothin et al. 1989. J. Cell Biol. 109:1621). The similarity between waves and growth cones raises the possibility that waves may represent a mechanism by which materials found in growth cones are delivered to the tips of developing processes.

Filopodia have been regarded as the sensory extensions of neuronal growth cones, and they are implicated in directing growth cones towards their targets. To study how filopodia themselves react to environmental cues, it is necessary to isolate them in isolation. We have isolated filopodia from growth cones on identified neuron B19 of the snail Helixasena. The cells were grown in conditioned culture media (L-15) for 18-24 hours. Subsequently, filopodia were mechanically transferred from the growth cone with a fine tipped micropipette. Many isolated filopodia retained their original shape for times exceeding one hour. In order to test the ability of filopodia to act as sensory structures, we asked whether isolated filopodia could respond to environmental stimuli known to affect growth cone morphology. Previous work has demonstrated that focal adhesion applied to growth cones and extracellular K+ concentrations dramatically alter growth cone morphology. Isolated filopodia show striking morphological changes in response to these stimuli: they either retract or extend, while a few showed no response. Also in accord with whole growth cone responses, isolated filopodia respond to the neurotransmitter serotonin.

Additionally, freeze fracture results demonstrate a high density of intramembrane particles on growth cone filopodia. Taken together these results suggest filopodia possess a complement of surface molecules including ion channels and specific neurotransmitter receptors that allow them to act as sensors of their environment.


Adult rat DRG neurons were dissociated and maintained in culture for up to one week. Relative intracellular calcium levels [Ca2+]i were imaged and measured using the calcium indicator dicye-3AM and confocal scanning laser microscopy (CSLM), at various times after plating (AP), either before, during, or after process regeneration and outgrowth. Cells were loaded in 5 mM CSAM for 35 minutes, transferred to a perfusion bath of oxygenated normal Krebs’ solution (with 2 mM Ca2+), and resting levels of [Ca2+]i were imaged with the CSLM in a frame-scan mode. Depolarization of cells via high (60 mM) in the bath resulted in elevated levels of [Ca2+]i, with a proportionally greater fluorescent signal seen in the nucleus and nucleolus 24 hours AP (during neurite outgrowth), compared to 5 hours AP, or more than 100 hours AP (after extensive process outgrowth). The nuclear signal was greater than that of the cytoplasm immediately after plating, but attenuated over time in the culture. The nuclear signal was less than the nuclear signal immediately after plating, increased and surpassed the nuclear signal from 12-48 hours AP, and then diminished again at times greater than 100 hours AP. Local bipolar electrode stimulation at 0 Hz, 1 second duration resulted in transient, reversible deviations in [Ca2+]i in the three cellular compartments. These electrophoretic changes could be repeated several times sequentially in most cells; occasionally cells responded with chronically elevated [Ca2+]i levels to the initial depolarizing current. High resolution imaging of the nucleus and nucleolus 24 hours after plating revealed discrete heterogeneity of the intranuclear fluorescent signal, suggesting the involvement of Ca2+ in nuclear mechanisms related to process regeneration and outgrowth.

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Growth cone motility is exquisitely sensitive to changes in intracellular calcium and electrical stimulation. We measured the kinetics of electrically evoked Ca2+ transients in mouse DRG growth cones and homoeostatic of Ca2+ during and after 15 min. trains of action potentials. Intrinsic Ca2+ (Ca2+ in Ca2+-free media) was not altered by chronic stimulation. In contrast, in neurons stimulated chronically (> 24 hrs.), electrically-evoked Ca2+ transients were slowed (t = 0.0005).

Intracellular Ca2+ recovered during stimulation (t = 0.048), and after terminating a 15 min. period of stimulation (t = 0.050). This may involve decreased Ca2+ removal or buffering mechanism. Acute Ca2+ removal or buffering mechanism would make growth cones from electrically active circuits less vulnerable to electrically-induced collapse. In addition, activity-dependent homeostas could have effects on other neuronal processes that are dependent on calcium.

543.14 CHRONIC EXPOSURE TO PICROTOXIN INCREASES AXOSOMATIC SYNAPSES AND FUNCTIONAL INHIBITION OF PURKINJE CELLS IN MOUSE CEREBELLAR CULTURES. R. Drake-Baumann 1, 2, A. L. Leiman 4, R. M. Herron 1, 2, K. T. Tarkkonen, 1, 2 and J. E. Stell. 3, 4. 1, 2, 3 Neurology, VA Medical Center and Dept. of Neurology, 4. Dept. of Physiology, University of California, Berkeley, CA and Good Samaritan Hospital, Portland, OR.

Organotypic cerebellar cultures of newborn mice were continuously exposed either to picROTOXIN (PTX) or to penicillin (PEN) in nutrient medium from explantation until 13-16 days in vitro (DIV) to examine whether chronic enhancement of neuronal activity would induce specific morphological or physiological changes. The spontaneous electrical activity of explants exposed to DIV revealed numerous units with slow discharge rates. The overall mean discharge rate for PTX treated explants was lower than for controls, while the overall mean discharge rate for PEN treated explants was slightly higher than controls and often revealed a tendency to bursting activity. Cortical stimulation produced a transient inhibition of spike discharge in all groups but the inhibitory effect was significantly enhanced in PTX treated explants. Explants exposed to PTX revealed a number of synapses on Purkinje cell (PC) somata (SPC), most of which appeared to be with basket cell terminals, while the number of axosomatic synapses present on granule cells of PEN treated explants did not differ from controls (2.2/PC).

The mechanism by which chronic exposure to PTX enhances synapse formation by basket cells may be independent of its inhibitory action on GABAergic transmission.

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544.1 ROLE OF NERVE GROWTH FACTOR (NGF) IN THE EARLY DEVELOPMENT OF QUAIL DORSAL ROOT GANGLIA (DRG). L. Yeo, I. Speights, and P. Bernd. Department of Anatomy and Cell Biology, State University of New York, Health Science Center, Brooklyn, NY 11203.

We have providing evidence that 125I-NGF binding to DRG, in vivo, is first seen at embryonic day 3.5 (E3.5; stage 23) in cytosol extracts of quail. Physiologically important high affinity NGF receptors appear to be present, because specific binding is competed by an excess of 125I-NGF at concentrations as low as 0.2 ng/ml (180 pM) and cross-linking with HSAB reveals that the high molecular weight 125I-NGF-receptor complex is present. These results are surprising since other labs have shown that avian DRG are unresponsive to NGF, prior to E5, at least with respect to axon growth. Our in vitro studies have examined the effects of NGF on neuron-enriched cultures prepared from E3.5 DRG. Cells were grown at a density of 2500/20 mm well in complete medium (MEM with 15% fetal bovine serum and 0.1% chick embryo extract) on acrylamide substrata. Preliminary results have shown that there were approximately 30 more neurons in the presence of exogenous NGF (100 ng/ml) after 24 hrs in vitro, as compared to cultures grown in complete medium in the presence anti-NGF (100 ng/ml), (boeblingen, Mannheim). The effect of NGF was also blocked by anti-NGF. We have also shown that anti-NGF has no toxic effect on the cultures; cell number remains the same when anti-NGF has been pre-absorbed with NGF. Cultures grown in complete medium with or without exogenous NGF have a similar number of neurotrophin-like substances in complete medium. Our current studies are aimed at determining whether the increase in cell number is due to a survival or mitogenic effect. Supported by a grant from the Deafness Research Foundation.

544.3 OVEREXPRESSATION OF NERVE GROWTH FACTOR IN EPIDEMISMS OF TRANSGENIC MICE CAUSES SKIN HYPERINNervation AND HYPERPLASTY OF TRIGEMINAL GANGLIA. K. Alberts, D. E. Wright, B. M. Davis. Departments of Pathology and Anatomy and Neurobiology, University of Kentucky School of Medicine, Lexington, KY 40536.

Nerve growth factor (NGF) is a target derived neurotrophic factor that plays a critical role in the survival and differentiation of neurons in the developing vertebrate nervous system. NGF deprivation in the embryo results in sensory degeneration whereas in the neonatal mice, lack of NGF leads to degeneration of neurons in the sympathetic nervous system. In cultures, NGF significantly enhances the survival of sensory and sympathetic neurons and neural crest derived cells. We have examined the role of NGF in neuronal survival by targeting the expression of NGF (125I-NGF (a gift from Dr. Edwards, UC] to the epidermis of transgenic mice. Mouse NGF cDNA expression was driven using the human keratin K14 promoter and enhancer sequences. K14 is an intermediate filament protein expressed in the basal cell layer of the epidermis and other stratified squamous epithelium. The increased levels of NGF resulting from expression of the K14-NGF transgene led to an increase in the density of innervation in the epidermal target tissue, hypertrophy of the trigeminal ganglia, and an increase in the number and size of primary afferents. These results demonstrate that in vivo the level of NGF in the target tissue controls cell survival and growth of primary afferents. Supported by NIH N2S5617 to BMD & AR40873 to KMA.


A series of novel nonpeptide ligands has been discovered that selectively displace NGF from its p75 binding protein (PD 90780). PD 90780 is characteristic of this series. It displaces 125I-NGF from the extracellular domain of p75 in a cell free system with an IC50 between 100-200 nM. PD 90780 also inhibits DBS croslinking of 125I-NGF to p75 but not to gp140(DH), suggesting that PD 90780 does not inhibit the binding of NGF to gp140(DH). Two 125I-NGF binding components were seen in whole PC12 cells in the presence of PD 90780. A large proportion of 125I-NGF binding was blocked by PD 90780 with an IC50 of approximately 200 nM. A smaller component of 125I-NGF binding was not displaced by PD 90780. The relative proportion of PD 90780 sensitive and resistant binding is similar to the proportion of p75 and gp140(DH) BP immunoprecipitated from native PC12 cells. Therefore, the PD 90780 sensitive binding of 125I-NGF in these studies may reflect binding of NGF to p75 while the PD 90780 resistant binding may reflect PD 90780 binding to gp140(DH).

Despite the ability to completely displace 125I-NGF from the p75 BP, PD 90780 does not block the ability of NGF to stimulate CHAT and MAP kinase activity in PC1 cells. PD 90780 also does not block the ability of NGF to promote survival of rat fetal SCG neurons in culture or inhibit the retrograde transport of NGF from the eye to the SCG in adult rats.


Nerve Growth Factor (NGF) binds to p75 and gp140 receptor proteins, signaling through the latter. PD90780 is a small organic molecule that displaces NGF binding from the extracellular domain of recombantly-expressed p75 in a cell-free system. In vitro autocrine NGF binding was performed using 125I-NGF in the presence of 100nM PD90780 at the distribution of binding sites determined from film and emission autoradiograms. In adult rat brain sections, NGF binding that was not displaced by PD90780 was observed on cell bodies in olfactory tubercle, nucleus accumbens, caudate-putamen, medial septum, diagonal bands, midline thalamic nuclei, cochlear nucleus, interpeduncular nucleus, spinal trigeminal nucleus. Unlabeled, lightly-labeled or heavily-labeled cell bodies were intermingled in all subdivisions of the trigeminal ganglia. Other ganglia were not examined. Neuronal labeling was observed in regions harboring labeled cell bodies as well as presumptive terminal fields of these cell bodies, i.e., cerebral cortex, basolateral amygdala, hippocampal formation, the spinal trigeminal tract and substantia gelatinosa. Spinal intersegmental rickets and a ventral tract to the central canal were also labeled. On postocciatal day (p.c.d.)12-16, labeled structures included trigeminal nuclei, superior cervical, dorsal root ganglia, spinal cord and regions surrounding whisker follicles. By p.c.d. 20, basal ganglia and forebrain labeling were apparent. In early cerebellar development, transient labeling of the molecular layer was observed. These observations are consistent with the hypothesis that NGF targets field innervation and maintains the differentiation state of neuronal subpopulations, e.g., cholinergic neurons. Work is in progress to determine if NGF binding sites that are not displaced by PD90780 represent p140(A). We appreciate the contributions of our Parke-Davis colleagues, J. Marks, K. Spiegel and T. Heoburn, who identified and characterized PD90780.


Nerve Growth Factor (NGF) is an endogenously expressed protein that is responsible for survival and maintenance of select neuronal populations. NGF has been shown to protect basal forebrain cholinergic neurons from atrophy due to age and damage. The effects of NGF on central cholinergic neurons, however, has not been rigorously studied. We report that continuous intracerebroventricular infusions of NGF into otherwise undamaged male rats increase mRNA and protein levels of acetylcholinesterase (AChE) activity in regions of the brain rich in cholinergic neuronal cell bodies: striatum, basal forebrain, and medial septum. Intentional damage to the striatum enhanced the ability of NGF to increase ChAT activity in forebrain cholinergic neurons. This effect of NGF developed gradually over the course of the infusion, reaching a peak following 7 to 10 days of administration. Additionally, intentionnal damage to the brain shifted the dose-effect curve forward suggesting that the damaged brain exhibits heightened responsiveness to NGF. A marked loss or atrophy of forebrain cholinergic neurons is one characteristic of Alzheimer's disease (AD). These results provide the cautious therapeutic use of NGF in AD as a means to prevent or reverse cholinergic neuronal atrophy. Because NGF does not travel far from the site of injection, however, procedures to ensure adequate delivery of NGF to target neuronal populations in the human brain.

Genetically modified cultured cells have been employed to express NGF in brain. We sought to determine if NGF expression in such cells could be modified by regulatory effectors. An 820 bp Pst I fragment containing the entire coding region of mouse NGF gene plus 5' flanking DNA was directionally cloned into the human metallothionein IIA promoter-driven expression vector pH6 (Invitrogen). This vector contains the zinc-inducible IIA promoter and SV40 polyadenylation signal, and utilizes the hygromycin B gene as the selectable marker. Recombinants were transfected into the rat glioma C6 cell line (calcium phosphate transfection). Transfectants were selected with hygromycin B (1000 µg/ml; 10-14 days) and colonies isolated using glass cloning cylinders. The transfection efficiency is 7.5 ± 107. Clonal isolates will be analyzed to determine gene dosage (genomic Southern blot), basal and induced levels of NGF mRNA (Northern blot), and intracellular and secreted NGF (ELISA). Transfectants screened to identify cell clones with minimal leakiness of the IIA promoter will be used as donor cells for transplantation into rat brain. Plt studies of C6 cell implants in rat brain do not show evidence of metastatic seeding or graft rejection.

544.9  CONSEQUENCES OF INCREASED NGF GENE EXPRESSION IN THE HEARTS OF TRANSGENIC MICE. A. Hassamani, M. Schlother, L. Field, M. Grisham and R.J. Fedde. fABen Eppen College of Medicine, Bronx, NY 10461 and Kranzler Institute for Cardiology, University of Indiana School of Medicine

To examine the role of nerve growth factor (NGF) in determining the extent of innervation of sympathetic neurons in a target organ, a transgenic mouse model has been created. The cardiac specific ß-myosin heavy chain (ß-MHC) promoter has been used to drive the expression of an NGF minigene (ß-MHC-NGF). ß-MHC is activated prior to the ontogenic development of cardiac innervation, becoming the expression of the cardiac ß-myosin heavy chain expressed in the adult rodent heart. Five independent ß-MHC-NGF founders were initially obtained, all of which expressed the transgene exclusively in the heart as demonstrated by RNase protection and reverse transcription PCR. These lines all exhibited an interesting albeit variable phenotype consisting of grosscardiomegaly and the presence of immature neural cells which appear to be infiltrating the epicardial region and particularly the aorto-ventricular groove. We have observed death (probable due to arrhythmia) in all transgenic lines. Increased NGF would be expected to cause sympathetic hyperinnervation and an attendant increase in cardiac cardiomere content. Examination of all transgenic lines reveals a significant increase in the level of cardiac tissue cardiomere content. The extent of cardiomegaly has been best correlated with the level of total cardiomeres. During propagation of the ß-MHC-NGF lines, we observed a decline in the penetrance of phenotype and expression of the transgene. Available data indicate that methylation of the transgene, within the ß-MHC promoter is responsible for the diminished expression of the transgene. Studies are currently underway to further characterize physiological and neurobiological issues related to this model.


Histidine residues 75 or 84 of mouse NGF were replaced with an alanine residue in single-mutant mice. Mutant genes were transiently expressed in COS-7 cells and proteins were partially purified with an NGF-specific, NHE monoclonal immunosaffron column. The bioactivity of each His mutant was compared with authentic NGF from mouse submaxillary glands by neurite outgrowth in PC12 cells, which showed about 5-10% less activity, while H6A showed far less activity than NGF. These results indicate that Hist 75 and 84 are both involved in the interaction with the receptor(s) and support previous chemical modification studies of His residues with diethylpyrocarbonate. However, the lower activity of these mutants could result from incomplete protein folding when transiently expressed in COS-7 cells.

Recombinant mouse NGF (rNGF) was isolated from insect cells infected with a recombinant baculovirus containing a prepro-NGF insert. Three purified forms of rNGF were separated on Mono-S chromatography and analyzed for biological activity in DRG and PC12 cell assays. Each form of rNGF differed from mature submaxillary gland NGF in that the C-terminal Arg-Gly dipeptide was not bound by the antibody. Receptor binding studies indicated that the NGF peptide treatment converted the three forms into a single form, identical to mature rNGF in structure and bioactivity. Thus, a single polypeptide of rNGF can exhibit distinct biological activities due to the C-terminal dipeptide. We suggest that C-terminal processing of NGF may be physiologically important. (Supported by NIH grant NS24380)

544.14  NEUROTROPHIN EXPRESSION IN THE DEVELOPING CHICK RETINA. X.-Y. Kao, *A.S. Gurner, J.M. Voigt, and T.R. Large. Dept. of Neuroscience, Case Western Reserve University, Cleveland, OH 44106

The biological actions of the neurotrophins in the developing nervous system are not limited to survival, but now appear to include the regulation of axon cell proliferation and commitment to a phenotype by post-mitotic cells. We have begun to examine these potential roles using the embryonic chick retina as a model system. Northern analysis of RNA from developing chick retina revealed that the neurotrophins and their receptors are expressed during the period of cell proliferation (E3-E7) and the subsequent formation of retinal layers. Transcripts for NT3 (E11), NTF3 and NT4 are present at E5, the earliest age tested. An increase in mRNA levels is observed at E13 and corresponds to the period of ganglion cell (RCG) dependence on target-derived factor(s) and maximal responsiveness of the RCG to neurotrophic factors. NT3 mRNA is present at E14 and is maximal by E16, but BDNF mRNA remains relatively low throughout development. NT3 mRNA has traditionally been shown to be present as early as E6. Thus, NT3 and NT4 may be ‘intrinsically’ regulators of retinal development while BDNF is a candidate ‘extrinsic’ factor supporting RGC survival.

In order to further examine neurotrophin actions we have begun to express recombinant neurotrophins in baculovirus. The coding sequence for the precursors of chicken NGF, BDNF and NT3 (differs from chick by one K substitution) were subcloned into the Bluebac transfer vector for color selection of recombinants. The use of linearized baculovirus DNA by the c.t.3 method identified recombinants. Finally, the adaptation of Hi-6 cells to suspension culture in serum-free medium yielded higher expression of recombinant factors compared to Hi-5 monolayer or 5i9 suspension cultures. Bioassays using PC12 cells and chick include H4 to properly process the precursor proteins to the active, mature forms. In addition, the 13 kD mature chicken NGF protein binds to the chick receptor against mouse NGF. These recombinant neurotrophins and the ongoing production of factor-specific, function blocking antibodies will allow us to manipulate neurotrophic levels and to test neurotrophin functions in vivo.

Supported by grants from the American Heart Association to X.Z. and M.T. from MSTP to ASG and NIH grants AG10053 to M.T. and D1989S to TRL.
544.14
The trk proto-oncogene has recently been identified as an important component of the nerve growth factor (NGF) receptor and has been implicated in high affinity binding of NGF. To begin investigating the regulation of the trk proto-oncogene (trk) during development, we have studied prenatal and postnatal expression in the rat, by in situ hybridization. Our results indicate temporal and spatial regulation of trk expression. At early embryonic stages (E13.5 and E14.5), trk mRNA was exclusively localized to spinal and cranial ganglia. In the central nervous system trk expression occurred at later developmental stages. During the first postnatal weeks (P1 and P15), highest levels of trk mRNA were observed in the hippocampus. However, in the adult CNS, expression of trk in regions such as the basal forebrain and piriform cortex was comparable to that in hippocampus. Our studies suggest that actions of NGF on various cell types during development may be modulated by temporal and spatial regulation of trk proto-oncogene expression. (Supported by NS 10259-Javitz Award and NIHF: HD23315)

544.15
NEUROTROPHIN RECEPTOR EXPRESSION IN THE DEVELOPING RAT EMBRYO. N.G. Carr*, S. Söderström and T. Enlund. Department of Developmental Biology, Uppsala University, Biomedical Center, S-751 23 Uppsala, Sweden and MBBCE*, GC 403, 1900 L Pampa, Argentina.
Expression of neurotrophin receptors was studied using antisense oligonucleotides specific for the low-affinity NGF receptor (LNGFR) and for the neurotrophin high-affinity receptors trkA, trkB and trkC. In situ hybridization was performed on sections of rat embryos at days E16, E20 and E22. The hybridization patterns were quantitatively analyzed in contact-exposed X-ray films using a video-based image processing system. The percentage of neuron area covered by silver grains in emulsion-dipped microscopic slides was also studied using this system. The results show distinct patterns of distribution for mRNA for each of the receptors. In the E16 embryo, LNGFR mRNA was highly expressed in the tegmental and dorsal root ganglia, in the ventral part of the spinal cord. Weaker labeling was found also in the retina. The trk oligoprobes intensively labeled the perinatal retina and, in the postnatal stage, the retinal and spinal cord. trkB was highly expressed in the telencephalon, retina, trigeminal ganglion and was abundant throughout the spinal cord. trkC expression was intense in the retina at E14. The same pattern was found at E20, although trkB labeling in the retina. Also at E22 similar patterns were found with an increased labeling of retinal neurons with the LNGFR probe. Our results show that neurotrophin receptors are expressed in neurons in a developmentally regulated pattern. The temporal-spatial expression of neurotrophin receptors may regulate the lophic response of a given neuronal population such as the retinal ganglion cells to a particular neurotrophic factor during development. (Supported by the Swedish Natural Science and Medical Research Councils, the Swedish Environmental Protection Agency and Concel-TWAS BC 90-098)

544.16
REGULATION OF p75NGFR AND p140trk EXPRESSION IN DEVELOPING SENSORY NEURONS IN RELATION TO INNERVATION AND CHANGES IN NEUROTROPHIC FACTOR RESPONSIVENESS. Sean Wyatt*, Luis Parada*, Susan O. Meakin* and Alan Davies*. 1St. George’s Hospital Medical School, London SW17 ORK, UK; NCFL, Fredrick, MD21701; 3Stanford University, CA4305.
When embryonic mouse trigeminal neurons are extending axons to their targets they survive independently of neurotrophic factors. As their axons approach their targets they display a transitory survival response to BDNF and NT-3 which is lost as the neurons become dependent on NGF for survival. Before acquiring NGF dependence, these neurons express low levels of p75NGFR and p140trk mRNA, which increase in the hippocampus and spinal cord between E16 and E18. Following establishment of NT-4/5 innervation, p75NGFR and p140trk mRNA increase in the hippocampus. At the stage of NT-4/5 withdrawal, p75NGFR mRNA declines, whereas p140trk mRNA increases. We are currently studying the effects of neurotrophic factors on p140trk mRNA expression in this system.

544.17
IMMUNOHISTOCHEMICAL DETECTION OF p75NGFR IN NEONATAL AND ADULT OLFACTORY NEUROEPITHELIA OF RAT. C. P. Turner* and J. R. Perez-Polo, Human Biological Chemistry and Genetics, Univ. Texas Med. Branch, Galveston, TX 77555-0652.
We have shown expression of the low affinity receptor for NGF, p75NGFR, on olfactory nerve body 19C. In neonates, we have observed p75NGFR-immunoreactivity (p75NGFR -ir) in the olfactory neuroepithelium (ONE) that was confined to a superficial band, superficial to the lamina propria (LP). A stain-free, single cell layer between the LP and band of p75NGFR -ir was very obvious at all neonatal ages. The absence of p75NGFR in this one cell layer suggests that only immature and/or native olfactory receptor neuron (ORNs) express p75NGFR. In adults, staining of the ONE was confined to the deepest layer, indicating that stem cells now express p75NGFR. However, the profiles displayed in neonates versus adults may be a reflection of the change in cell dynamics that takes place in the ONE as the animal matures. Within the LP, we observed staining of the olfactory fasicles at all ages. We believe glial cells are responsible for this staining, Not all fasicles were stained, perhaps because there is a mixed population of mature and immature ORN axons. The maxillary ONE is one of the last to develop throughout life and expression of p75NGFR in both adults as well as neonates implies that neurotrophic support is required at all times. Supported in part by MDES Grant #18708.

544.18
The p75 neurotrophin binding protein (low-affinity NGF receptor) is a transmembrane receptor for NGF, BDNF, and NT3, with no known signal transduction activity. In previous studies of rat and primate retina, p75 immunoreactivity appeared to be confined to Muller glia, with rare p75-positive retinal ganglion cells (RGC) in adult rat. One report of p75 in the optic nerve fiber layer of developing primate retina suggested p75 involvement in the growth of RGC axons. As a part of a study of neurotrophin receptors in the human retina, we have examined the distribution of p75 in normal embryonic human retina, normal adult human retina, and regenerating adult human retina. Adult human donor eyes were a generous gift of the Mid-America Eye and Tissue Bank, St. Louis, MO. Retinas were either cultured on a monolayer of transformed rat Schwann cells or prepared for cryostat sectioning. Sections of embryonic human retina were prepared as part of an approved study at the University of Miami. Sections and cultures were immunostained with a monoclonal antibody (ME20.4) directed against human p75. p75 appeared to be confined to Muller glia in the adult human retina. In embryonic retinas at 6 weeks of age, p75 was evenly distributed in the retina, with no enhancement of p75 staining in the RGC fiber layer. ME20.4 staining did not detect p75 on adult human retinal structures regenerating after retinal detachment surgery. We conclude that human retinal ganglion cells and their axons do not express significant amounts of p75 protein in the normal adult or the early embryo. (Supported by Warner-Lambert Co. and The Miami Project.)
544.19 RAS p21 PROTEIN PROMOTES SURVIVAL AND NEURITE OUTGROWTH OF NGF-DEPENDENT HUMAN EMBRYONIC NEURAL CYTOSKELETAL DERIVATIVES IN VITRO. V. Silani*, A. Markwe, G. Sciaracca*, R. Heymnan* and G.D. Rosato*. "Diino Foundation, Nervosco University,孑sfield, 1205, Switzerland; 3. Leibnitz Institute for Neurobiochemistry, Ruhr-Universität Bochum, D-4060 Bochum; and Neurologische Universitätsklinikum, Klinikum Großhadern, Ludwig-Maximilians-Universität, Munich 70, Germany.

In previous studies we demonstrated dose-dependent survival and neurite outgrowth induced by the ras p21 protein after microinjection into cultured chick embryo neurons. To determine whether similar effects could be induced in human cells, we introduced the oncogenic form of ras p21 into the cytoplasm of cultured human neural crest derivatives (11th to 12th gestational weeks) and human chromaffin cells, dorsal root ganglion (DRG) and sympathetic neurons. We verified that these cells are dependent on nerve growth factor (NGF) for survival and neurite outgrowth in vitro. In DRG neurons, ras p21 increased promoted survival and neurite outgrowth comparable with NGF (84% vs. 92%, respectively). Sympathetic neurons in the chick embryos were demonstrated to be unresponsive to ras p21. Intriguingly, this molecule promoted survival in human sympathetic neurons, albeit less strongly than NGF (67% vs. 94%). The effect of ras p21 on purified human chromaffin cells was indistinguishable from NGF (41% vs. 40% neurite outgrowth). These results are compatible with the involvement of ras or ras-like proteins in the intracellular transduction of NGF in human neural crest derivatives and suggest species-specific differences in neurotrophic signal transduction.

544.21 NEUROTROPHINS AFFECT THE ISTHM-OPTIC NUCLEUS IN CHICK EMBRYOS: CELL DEATH ENHANCED BY NGF IN VIVO. C.S. van Barlheid*, Y. Kinoshita*, and M. Bothwell. Department of Physiology & Biophysics (S&40), University of Washington, Seattle, WA 98195.

The isthmo-optic nucleus (ION) is the source of centrifugal innervation of the retina. Neurons in the ION express p75 neurotrophin receptors during the period of normal developmental cell death (E13-17, von Barlheid et al., 1991, J. Comp. Neural. 310: 105-129). To determine which neurotrophin supports ION development, we tested NGF, BDNF, and NT-3 (1-10 ng/ml) on ION neurons in primary culture. ION neurons were identified by intracellular injection of the retrograde transport of the retrograde tracer Dil, and labeled cells were plated from embryos at E11, E12 and E13. BDNF increased survival and neurite extension of ION neurons. The survival effect was most pronounced on neurons from E12 embryos, but still significant for ION neurons from E12/13 embryos. Neither NGF nor NT-3 increased survival rates. To determine effects of neurotrophins on ION neurons in vivo, we injected 75-900 ng of NGF in the eye daily from E10-15. This treatment enhanced cell death in the ION by 25-72% in the embryos, but not at later ages. Injections of vehicle only did not increase cell death. The negative effect of NGF on developing ION neurons may be due to competition at the level of the neurotrophin receptor. NGF may act as a competitive antagonist, possibly to BDNF, thus preventing trophic action. Competitive interactions among the neurotrophins may play a role in the specificity of target innervation and the regulation of neuronal survival. Supported by NIH grants NS 88990, 23343 and 2982.


vGF is a neural immediate-early gene product which is rapidly and selectively induced by neurotrophic factors (e.g., nerve growth factor, NGF). The role of vGF in neurotrophic factors in P12 cells. We have determined the anatomical distribution of vGF mRNA in the P5 rat brain by in situ hybridization using 5'-32P-labeled antisense probes to vGF mRNA. Selective labeling of limbic and paralimbic cortex, including cingulate, retrosplenial, entorhinal, and piriform cortices, was noted in contrast to a distinct lack of signal in frontoparietal, occipital or temporal regions. Strong labeling was also seen in olfactory bulb, the hippocampal formation (particularly CA1 and dentate), amygdala, basal forebrain, striatum, nucleus accumbens, lateral geniculate, gelatinous and ventroposterolateral/ventroposteromedial thalamic nuclei, septal nuclei, inferior colliculus, cerebellum, and portions of the pons and medulla. A previous study of whole brains found vGF mRNA to first be detectable at embryonic day 18 (E18), peak at P4, and decrease to intermediate levels in the adult (Salton et al., 1991). An ongoing in situ hybridization studies of animals from embryogenesis through adulthood will determine the ontogeny of vGF expression in specific brain structures.


We investigated whether purified alpha and gamma subunits from murine 75 complex could form a complex with either mature mRN or precursor forms of mRN. The mature and precursor forms of mRN were isolated by classical purification techniques (Schmeieter, et al., 1992) J. Neurochem, in press and unexploited experiments, respectively). Multiple forms of the precursor were observed as two major groups of bands on SDS-PAGE. 26 kDa and 18 kDa. A more complete characterization of the precursor forms will be presented. We observed complex formation with several forms of mature mRN as well as precursor processing to mature mRN and these processing intermediates were either alpha or gamma subunit. Two different forms of 75 complex were found each form of the precursor could be resolved into four bands in the region of 13 kDa on SDS-PAGE, corresponding to a mature NGF. The activity of the processed precursor and its resulting processed product was compared using PC-12 cell-based neurite extension and chick dorsal root ganglion cell survival assays. Processed precursor yielded mature mRN showing full activity while precursor itself exhibited minimal activity.

545.1 ASTROCYTES PROTECT DOPAMINERGIC NEURONS AGAINST TOXICITY PRODUCED BY 1-METHYL-4-PHENYLPIRYRIDINE (MPP+) AND 6-HYDOXYDOPAMINE (6-OHDA) IN CULTURE. T.H. Park and C. Myelinogen. Dept. of Neurology, Mt. Sinai School of Medicine, New York, N.Y. 10029.

We studied the trophic effect of astrocytes upon dopamine (DA) neurons to examine whether or not glia can modify the toxicity produced by the dopaminergic toxins, MPP+ and 6-OHDA. Glial cultures, enriched in astrocytes, were prepared from the striatum or mesencephalon of newborn rats in a serum-free medium and 24 hr before use were switched to chemically defined medium. Mesencephalic cells from embryonic day 14 rats were plated on either polystyrene coated 30mm dishes or on dishes with a confluent layer of astrocytes. MPP+ (10-8 to 10-4 M) was applied for 4-8 days in vitro (DIV) and 6-OHDA (100ugM) for 45 min at DIV. Neurotoxicity was assessed at DIV by MDA uptake and morphometric analysis of both MPP+ and 6-OHDA treated cells. The extent of neuronal damage and neuronal survival. We found that the presence of either striatal or mesencephalic glia modified the extent of neuronal toxicity produced by both MPP+ and 6-OHDA. Mouse DA neurons were maintained in cultures with approximately 5% of control after treatment with either MPP+ or 6-OHDA, while DA uptake was 15% of control after MPP+ and ~60% of control after 6-OHDA treatment. Similarly, the number of surviving TH + cells was between 20-40% of control after MPP+ or 6-OHDA treatment in neuronal cultures, compared with ~90% of control in mixed cultures. Our results show that the presence of glia can protect DA neurons against uptake reduction and cell loss produced by both toxins. However, the glial protective effect against 6-OHDA toxicity is greater than against MPP+.

545.2 TYPE 1 ASTROCYTE CONDITIONED MEDIUM PROTECTS SUBSTANTIA NIGRA DOPAMINE NEURONS AGAINST EXCITOTOXIC DAMAGE. L.B. Black and C.F. Greys. Dept. Neuroscience and Cell Biology, UMDS-J.

R.W. Johnson Medical School, Piscataway, NJ 08854.

Previous work from our laboratory has shown that Type 1 astrocytes from the substantia nigra selectively enhance the survival of substantia nigra dopamine neurons in dissociated cell culture. Further, conditioned medium from Type 1 astrocyte cultures (CM) is able to block neurotoxic effects (O'Malley et al., 1992). To determine whether the CM effect on survival could be extended to other types of astrocytes, CM from either sense, New York, N.Y. was CM. CH. Schmeieter. Genentech, Inc., South San Francisco, CA 94080.

We investigated whether purified alpha and gamma subunits from murine 75 complex could form a complex with either mature mRN or precursor forms of mRN. The mature and precursor forms of mRN were isolated by classical purification techniques (Schmeieter, et al., 1992) J. Neurochem, in press and unexploited experiments, respectively). Multiple forms of the precursor were observed as two major groups of bands on SDS-PAGE. 26 kDa and 18 kDa. A more complete characterization of the precursor forms will be presented. We observed complex formation with several forms of mature mRN as well as precursor processing to mature mRN and these processing intermediates were either alpha or gamma subunit. Two different forms of 75 complex were found each form of the precursor could be resolved into four bands in the region of 13 kDa on SDS-PAGE, corresponding to a mature NGF. The activity of the processed precursor and its resulting processed product was compared using PC-12 cell-based neurite extension and chick dorsal root ganglion cell survival assays. Processed precursor yielded mature mRN showing full activity while precursor itself exhibited minimal activity.
545.3

SUBSTANIA NIGRA TYPE I ASTROCYTES ELABORATE A SOLUBLE FACTOR THAT PROMOTES DIFFERENTIATION OF NEURON SUBTYPES.


We have previously demonstrated that local glia, specifically Type I astrocytes, selectively increase substantia nigra (SN) dopaminergic neuron survival. However, the mechanism of action remains unclear. To determine whether effects are elicited through diffusible agents, partially purified Type I astrocyte conditioned medium (CM) was tested on embryonic day 16 rat SN dissociates. After 3 days of exposure to CM, DA neuron number was monitored immunocytochemically with antibody to tyrosine hydroxylase (TH), the DA biosynthetic enzyme. CM increased TH⁺ cell number 2-fold, suggesting that a soluble factor(s) promoted neuron survival.

Neurons cultured in serum free medium are known to contain few, but detectable numbers of glia. To determine whether CM affected neurons directly, or indirectly through glia, cultures were labeled with antibody against the glial marker, glial fibrillary acidic protein (GFAP). CM increased GFAP⁺ cells 2-fold, implying that CM may affect DA neuronal survival indirectly. To test this possibility, cultures were exposed to a concentration of the glitoxin α-amino adipic acid that eliminated detectable GFAP⁺ cells. Under these conditions, CM still elicited a 2-fold increase in TH⁺ cells. Our observations suggest that CM directly enhances substantia nigra DA neuron survival, in culture. (Support:NIH HD 23135, Javits:NS 10259.)

545.4

TGFα SELECTIVELY INCREASES DOPAMINERGIC CELL SURVIVAL IN VENTRAL MESENCEPHALIC HUMAN CULTURES. T. Akaoka, E. Kato, and F. Hattori. Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90009.

Transforming growth factor α (TGFα), a mRNA and protein have been localized to rodent brain (Wilcox and Derynck 1988; Fallon et al., 1990). Based on its high expression in the striatum, TGFα has been proposed as a trophic factor for dopaminergic neurons. Thereby we characterized the actions of TGFα on E15 fetal rat cultures of dopaminergic neurons. Addition of TGFα (10ng/ml) for 1 day to low-density mesencephalic cultures selectively promoted dopaminergic cell survival after 4 days as measured by an increase in the number of tyrosine hydroxylase (TH) immunopositive cells. These cells represent only 1% of the total neuron number. In contrast, the total number of neurons (neuron specific enolase, protein immunoactive) was unaffected. The effect of vinculin immunopositive astrocyes was elevated by 15% above control. Dopamine (DA) uptake and TH activity were also enhanced approximately 100% and 45% above control, respectively. In cultures lacking a polycationic substrate, TGFα dramatically amplified neuronal adhesion to astrocytes. This adhesion was blocked by the anti-motile agent, cytoxin arabinoside. Two other growth factors, epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1), stimulated DA uptake to a lesser degree than TGFα. EGF shares the same receptor with TGFα, and in combination the two did not show additive effects, while the EGF-induced elevation was additive with TGFα. Investigation of possible actions of endogenous neurotrophins with KS25b did not alter TGFα's effects on DA uptake or neuron-astrocyte adhesion. Ciliary neurotrophic factor was ineffect in altering control or TGFα or EGF uptake. These findings indicate that TGFα selectively promotes survival of dopaminergic neurons and that this effect is probably mediated by glial cells.

545.5


We have recently described a primary culture of mesencephalic dopaminergic neurons from human fetal brain that is 100% TH⁺. This culture shows between 4 hr and 10 days in culture (Soc. Neurosci. Abs 17: 982, 1991; Brain Res. In press). Progressive neuronal death occurs, and 14 days in culture (DIV14), <10% of all neurons survive. Those TH⁺ cells that survive, however, develop extensive, elaborate, neurofilament positive (NF+) dendritic arborizations, at DIV4 when the astrocytes are removed, become confluent, providing an astrocyte feeder layer (AFL) for cell growth. When the cells were grown on poly-D-Lysine, and seeded at 5.0 x 10⁵ cells/cm², or less, 99% of the neurons died by DIV5. However, when grown on an AFL, even at 1000 cells/cm², neuronal survival was >95% even at DIV14. The results suggest that confluent astrocytes produce a factor that promotes dopaminergic cell survival in culture. Preliminary results suggest that the conditioned medium from the DIV0 to DIV4 cultures also promotes dopaminergic neuronal survival. Extracts from astrocytes of DIV4 to DIV21 are also being tested. Experiments are in progress to identify and isolate the putative neuroprotective factor(s) (NTF) for dopaminergic neurons. Antibodies to several proposed NTFs for dopaminergic neurons did not inhibit dopaminergic cell rescue by the AFL or the conditioned medium. Isolated packets of cells develop in some cultures, all of which are TH⁺, suggesting that TH⁺ cell clones may develop in the cultures spontaneously. In addition, in some cultures, some TH⁺ cells developed elongated neurites that were devoid of varicosities, suggesting the existence of a specific, varicosity-inducing factor.

545.7

IN VIVO EXPRESSION OF PDGFRα IN PHENOTYPICALLY DEFINED GLIA. J.A. Ellisop and J. de Vella. Laboratory of Biomedical and Environmental Science, Dept. of Anatomy and Cell Biology, and the Mental Retardation Research Center, NY, Los Angeles, CA 90054.

Growth factors such as basic fibroblast growth factor and platelet derived growth factor (PDGF) can modulate certain influence migration, proliferation and differentiation. Recent studies have demonstrated the presence of PDGFRα receptors (PDGFRα) in the rodent brain (Schachner et al, 1990; Fraker et al., J. Neurosci., 11:828, 1991; and 31:92) and on cells of the OL lineage in vitro (McKinnon et al. Neuron.; 1990; Hart et. Dev. 102: 1869). No study, however, has demonstrated the presence of PDGFRα on phenotypically defined cells in vivo.

We used combining immunocytochemistry and in situ hybridization, PDGFRα mRNA in cells in both the gray and white matter of the neonatal rat mesencephalic cortex. We carried out a spatial and temporal analysis of PDGFRα mRNA expression in the rat forebrain on postnatal days 1.3, 6, and 9. PDGFRα message is detected in cells which are immunoreactive for vimentin, GD3, A2B5, and satellite astrocyte. In contrast, cells which are immunonegative for GAP-43, neuronal fibrillary acidic protein (GFAP), and 48 kD neur Filament protein (NF) do not express a detectable message. In vivo studies are important not only to verify in vitro data, but also to understand the cell-cell interactions that occur only when the cytoarchitecture is maintained. Our results support the in vivo data which suggest that: 1) PDGFRα is expressed by immature OL; 2) GD3+ and A2B5+ immature OL have the potential to respond to PDGF, and 3) GD-OL, GFAP+ astrocytes, and NF+ oligodendrocytes do not respond to PDGF.

Supported by NICHD and DOE.

545.8

GRAFTED SCHWANN CELLS AND INFUSION OF A SCHWANN CELL-DERIVED GROWTH FACTOR (DNTF) ENHANCE MORPHOLOGICAL RECOVERY IN THE DAMAGED ADULT RAT DOPAMINE SYSTEM. T.J. Collin,* P.N. Martin, B.A. Maguire, and L.R. Springer. Dept. Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14621; and Dept. Neurology, Hofstra University School of Medicine, Philadelphia, PA 19102.

We have previously demonstrated that co-cultured Schwann cells, or a partially purified fraction of conditioned medium from peripheral nerve Schwann cell line, denoted as dopaminergic neurotrophic factor (DNTF), promote survival and neurite outgrowth of cultured embryonic rat mesencephalic dopaminergic neurons (Colburn et al., J. Neurosci., 11:828, 1991; and 31:92) and on cells of the OL lineage in vitro (McKinnon et al. Neuron.; 1990; Hart et. Dev. 102: 1869). No study, however, has demonstrated the presence of PDGFRα on phenotypically defined cells in vivo.

We have used combining immunocytochemistry and in situ hybridization, PDGFRα mRNA in cells in both the gray and white matter of the neonatal rat mesencephalic cortex. We carried out a spatial and temporal analysis of PDGFRα mRNA expression in the rat forebrain on postnatal days 1.3, 6, and 9. PDGFRα message is detected in cells which are immunoreactive for vimentin, GD3, A2B5, and satellite astrocyte. In contrast, cells which are immunonegative for GAP-43, neuronal fibrillary acidic protein (GFAP), and 48 kD neur Filament protein (NF) do not express a detectable message. In vivo studies are important not only to verify in vitro data, but also to understand the cell-cell interactions that occur only when the cytoarchitecture is maintained. Our results support the in vivo data which suggest that: 1) PDGFRα is expressed by immature OL; 2) GD3+ and A2B5+ immature OL have the potential to respond to PDGF, and 3) GD-OL, GFAP+ astrocytes, and NF+ oligodendrocytes do not respond to PDGF.

Supported by NICHD and DOE.
545.9
An Assay for Detecting Schwann Cell Secretory Activity in Vivo. Danilo, J.K., K. Croissant, A. Smith, T. Yoes, S. Gibson and M. Powell. Department of Veterinary Anaesthesia, Louisiana State University, Baton Rouge, Louisiana and Department of Neurology, Scripps Research Institute, La Jolla, CA.
Schwann cells have broad contributions to development, regeneration, and tumor formation. In many situations, activity involves the coordinated production and secretion of various factors and neurotransmitters. In our assay, Schwann cells are co-cultured with fibroblasts to form a mixed monolayer. The Schwann cells secrete factors that stimulate fibroblast proliferation, which was quantitated by a colorimetric assay. The results indicate that Schwann cells are capable of producing a variety of factors that stimulate fibroblast proliferation.

545.10
TRANSFORMING GROWTH FACTOR ALPHA IS PRESENT IN DEVELOPING BRAIN AND A POTENT ASTROCYTE MITOGEN. Kenneth R. Hult* and Qi-Xiao Yue. Harbor-UCLA Medical Center, Dept of Pediatrics, Torrance, California.
Transforming growth factor alpha (TGFα) is a potent mitogen for astrocytes and other cell types. In the developing brain, TGFα is expressed in a variety of cellular and subcellular compartments. The expression of TGFα is regulated by a number of factors, including neuronal activity, microglial stimulation, and the presence of extracellular matrix components. The role of TGFα in the development of the brain is not yet clear, but it is likely to be important in the regulation of astrocyte proliferation and differentiation.

545.11
ESTRADIOL REGULATES S-100B mRNA EXPRESSION IN CULTURED RAT ASTROCYTOMAS. D.A. Hinkle, D.C. Hill, P.J. Yanovsky, S.R. Max, and P.M. Wise. Department of Physiology. University of Maryland School of Medicine, Baltimore, MD 21201.
Estradiol is implicated in reproductive senescence of the hypothalamus, a brain region which becomes markedly luteinized with increasing age and in response to treatment with estrogens. (Biof. Repro. 42:21-8, 1990). S-100B is a galactosylated protein which promotes the growth and development of neurons and glial cells, and is reported to be elevated in reactive astrocytes in gliotic brains. The aim of this study was to determine if and to what extent estradiol regulates S-100B mRNA expression in cultured rat astrocytes. Primary astrocytes were cultured from various brain regions of rats and were transfected with a reporter containing the lacZ gene and treated with estradiol. S-100B mRNA levels were decreased by estradiol treatment, suggesting that estradiol has a regulatory role in the expression of S-100B in astrocytes.

545.12
IN VITRO DIFFERENTIATION OF THE BASAL FOREBRAIN CHOLESTEROL METABOLIC FROM THE TONGUES IN MICE. M. Okai, S.M. Coetz, M. Trofa, T. Letitia, V. Del Fino Peace. Int. di Fisiologia Umana, Fac. di Medicina, Univ. di Bari, Italy; 70024; Consorzio di Ricerca D'ECOM, Bari, Italy; 70020.
The tongue is a unique organ that produces and secretes a variety of substances, including saliva and other secretions. The tongue is also an important organ for the production of cholesterol and other lipids. In this study, we investigated the differentiation of the basal forebrain cholesterol metabolic from the tongue in mice. We found that the tongue in mice produces a higher level of cholesterol than the basal forebrain. This suggests that the tongue may play a role in the production of cholesterol and other lipids.

545.13
To study astrocytic responses associated with spinal cord regeneration in adult rats, we used a model of spinal cord transection (Brain Res. Bull., 22:71-79, 1989) was used to produce focal spinal cord injuries. The lesions were characterized by a variety of biochemical and morphological changes, including increased levels of IGF-I and vimentin. The results indicate that astrocytes respond to spinal cord injury by expressing IGF-I and vimentin, which may play a role in the regeneration process.

545.14
Activity-dependent neurotrophic factor (ADNF) is a gliad-derived protein that regulates the survival and maturation of neurons. The ADNF receptor is a vasoactive intestinal peptide. ADNF has been purified to homogeneity and has been shown to increase the survival of neurons derived from spinal cord, cerebral cortex and hippocampus at concentrations <1 PM. Physical properties (16 KD, pi 6.1) and partial sequence data identified ADNF as a novel, neutralizing antisera. Neutralizing antisera was obtained by serial injections of ADNF into mice. In cerebral cortical or spinal cord cultures, ADNF antisera (1:1000) decreased neuronal counts by 50-65% of control after five days. Co-culture of purified ADNF prevented neuronal cell death associated with the antisera. Control antisera had no effect. These data support the conclusion that ADNF is important to the survival of a subset of cortical and spinal cord neurons.
VASOPRESSIN INDUCTION OF THE IMMEDIATE EARLY GENE, zf7-268, IN CULTURED HIPPOCAMPAL GLIAL CELLS.

R.D. Brinton*, K. O'Neil*, P. Kim*, S.S. Schreiber,

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The neural peptide, vasopressin (AVP), has been associated with growth responses in both neural (Brinton & Graurner, 1987, Brinton, 1990) and nonneural cell types (Rosenburg, 1979). The immediate early gene (IEG), zf7-268, is one of several genes induced in response to growth factors (Bartel et al., 1989). Because AVP affects cell growth, we investigated the influence of AVP on induction of zf7-268 expression in cultures of hippocampal neurons and glial cells. Hippocampal cells from E18 rat pups were cultured on polylysine-coated chamber slides in serum containing medium. Following 3 days in culture, cells were exposed to 10, 100, 500 or 1000 nM AVP or other peptides for 15 or 30 min. Paraformaldehyde fixed cells were treated with TEA (1:100) and hybridized with a 35S-labeled zf7-268 cRNA probe. Slides were dipped in NTB-2 emulsion, exposed at 4°C for 3-4 weeks and analyzed using a BioQuant image analysis system. Results of those studies showed AVP induction of zf7-268 in glial cells that was dose and time dependent. Greater grain density was observed in cells treated with 10 or 100 nM AVP whereas grain densities in glial cells treated with 500 or 1000 nM AVP were not as intense. Maximum induction of zf7-268 was observed in glial cells exposed to the AVP for 15 min. AVP induction of zf7-268 was IEG and specific in that this was not induced by AVP and AVP induction was specific to glial cells and not observed in neurons. Experiments to determine receptor specificity indicated that AVP induction of zf7-268 is mediated via a V1 receptor since the specific V1 antagonist Phe9-vasopressin at a concentration of 10 nM inhibited zf7-268 expression. Supported by NIH grants MH45036 and SO1 RO3 HL to R.B. and NS13353 to S.S.

BNDN and tbr2 mRNA REGULATION FOLLOWING SELECTIVE DEAFFERENTATION OF ADULT RAT HIPPOCAMPAL NEURONS.

M. Dugash-Djedovic, J. R. Day, D.A. Lukasch and F. Helt, Neurosurgery Center, University of Southern California, Los Angeles, CA 90033.

We have previously suggested that BDNF may participate in the synaptic remodeling responses observed in the adult rat hippocampus following the administration of systemic kainic acid (Dugash-Djedovic et al., Neurop., 47, 303-315, 1992). In order to further investigate the possible role of BDNF in neuronal plasticity in the adult, we used in situ hybridization histochemistry to examine changes in the transcriptional expression of BDNF in adult rat hippocampus following the administration of kainic acid. BDNF mRNA was localized in the dentate gyrus and hilus region on day 3, with elevated expression in the contralateral DG and control levels were observed at day 7. tbr2 mRNA expression was elevated non-neuronal populations of the septal aspect of the ipsilateral dentate molecular layer at 3 days following kainic acid administration. Unilateral destruction of dentate granule cells by colchicine resulted as a loss of expression in the ipsilateral DG and a pronounced increase in BDNF mRNA in the ipsilateral CA1 pyramidal layer and hilus region on day 3, with slightly elevated expression in the contralateral DG and CA3 at 10 day lesion. tbr2 mRNA expression was increased along the entire septotemporal portion of the ipsilateral dentate molecular layer from 102 day lesion, with elevated expression in areas adjacent to the CA1, CA3, and DG regions at 10 day following lesion. In summary, the expression of BDNF mRNA was increased at neuronal sites of axonal projections of deafferented areas. while altered expression of tbr2 was localized to apparent non-neuronal populations adjacent to denervated target regions. This finding suggests that BDNF and tbr2 may participate in neuronal plasticity in the adult rat brain following injury. Alternatively, the more delayed increases in tbr2 mRNA expression suggest a coordination with neurogenerative processes.

IMMUNOREACTIVITY TO MOTORNEUROTROPIC FACTOR I (MNFI) IN TOWERS OF ARKANSAS AND RHODE ISLAND. REINERVAIATION. F. Rem, R.W. Chan, W.B. Yu and T.C. Gu, Dept. of Anat., Univ. of Arkansas for Medical Sci., 4301 W. Markham St., Little Rock, AR 72205.

Monoclonal antibody raised against MNF, a 35 kD muscle-derived motoneuronal trophic factor, was used to study its regional localization in the tongue following denervation and reinervation. The right hypoglossal nerve of female Sprague-Dawley rats 2-3 months old was transected under anesthesia. Rats were perfused with 4% paraformaldehyde in PBS at 4°C, 1,3,5, and 2,5 post-transsection. Tissue was embedded and cut transversely for immunocytochemical procedures. Positive immunoreactivity was observed in the site of muscle fibers and in the axons of many large, myelinated nerve fibers on both sides of the tongue. The expression of MNF was dramatically enhanced following denervation. Monoclonal antibodies to MNF recognize the MNF protein in muscle fibers and in the axons of many large myelinated nerve fibers on both sides of the tongue. It has been postulated that MNF is a potent chemotactic factor for regenerating nerve fibers. The expression of MNF was significantly increased - this message was not detectable in contralateral control animals. These data indicate that axotomy, facial motoneurons and rubrospinal neurons may be responsive to BDNN and NT3. (Supported by MRC)
WITHDRAWN

546.7


The present study evaluates the long-term effects of neural lesions on the differentiation of the skin in postnatal oposen pup. Lesions of dorsal root ganglia A5 or spinal cord produce acute effects in the skin including hyperplasia of the epidermis, hyperkeratinization of the dermis and epidermis, and suppression of hair formation in the first week following neural lesions. By the second week precocious hair (more mature than expected) are present often with significantly abnormal shape and direction of the shaft relative to the skin surface. By three weeks the skin appears grossly normal and all abnormalities are visible to the eye. However, on studying serial sections the residual lesions of neural tube, DRG, and neural arches confirm the prior neural lesion. The only microscopic abnormality in the skin is the presence of isolated abnormal hairs in terms of shape and direction. These findings indicate the extent of trophic interaction of affereht nerves and skin and that afferent nerves control the differentiation of their targets in skin as well as muscle (Zelena, 1957).

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546.8


Although BFGF, like NGF, appears to be trophic for cholinergic neurons of the basal forebrain, its effects on cholinergic fibers have not been demonstrated. In rats with unilateral partial fimbrial transections, chronic administration of BFGF (10 mg) or NGF (1 μg) resulted in increases of ChAT activity in the lesioned hippocampi of similar magnitude (39 and 46%, respectively) compared to that of a control group. ACh content, basal and evoked ACh release were significantly reduced by 50%, 40%, and 53%, respectively. However, whereas only modest increases in these measures (16-18%) in the BFGF-treated group compared to the control were observed, marked enhancements in the NGF-treated rats were apparent. Thus, in the NGF-treated group, ACh content, basal and evoked ACh release in the lesioned hippocampi were augmented by 67%, 45%, and 51%, respectively. In addition, no synergy between BFGF and NGF was observed, since in rats receiving combined administration of BFGF and NGF, hippocampal cholinergic parameters were not markedly different from those measured in rats treated with NGF alone. Moreover, the binding of [3H]donepezil to lesioned hippocampi was similar in the lesioned hippocampi of the NGF-treated group and moderately enhanced compared to either the control or the BFGF-treated group. Thus, in conclusion, the limited effectiveness of BFGF in counteracting lesion-induced reductions of cholinergic function indicates that trophic factors may not be the treatment of choice in reversing the cholinergic deficit associated with certain neurodegenerative diseases.

546.9


Recent pharmacological studies have shown that BDNF increases ChAT activity in cultured septal cells suggesting that BDNF may be effective in stimulating the expression of the cholinergic phenotype in the adult brain. The present study determined the effects of chronic administration (ICV, 1.4 μg qod for 21 days) of recombinant human (rh) NGF or rhBDNF on hippocampal cholinergic function measured in vivo in adult rats with partial fimbrial transections. Chronic fimbrial transections reduced hippocampal high affinity choline uptake by 54%, ChAT activity by 41%, and ['3H]ACh synthesis by 63%. Chronic rhBDNF treatment failed to enhance the levels of these cholinergic parameters. In contrast, chronic treatment with rhNGF increased high affinity choline uptake by 112%, ChAT activity by 41%, and ['3H]ACh synthesis by 63%.

Chronic rhBDNF treatment, but not rhNGF treatment, significantly enhances markers of presynaptic hippocampal cholinergic function following partial fimbrial transections. Lastly, chronic treatment with either rhBDNF or rhNGF produced significant reductions in body weight (23-26 g/g) over the 21 day treatment schedule. Thus, although rhBDNF does not effect central cholinergic function it does seems to interact with CNS neurons to regulate weight gain. The results of the present study suggests that NGF will be more effective than BDNF in counteracting the cholinergic deficit associated with the cognitive decline observed in Alzheimer's disease.

546.6

SPINAL INJURY ALTERS CIRCULATING NEUROTROPHIC ACTIVITY. F. Reisen*, C. L. Lu, J. R. Johnson, R. D. Linden, O. Ninkic, L. Ray, C. B. Shields, L. Wang, G. Yenke and Y. P. Yang. School of Medicine, Neurobiology, Orthopedic Surgery, and Surgery (Division of Neurosurgery), University of Louisville School of Medicine, Louisville, KY 40292.

It is likely that trophic agents play a key role in mediating neuroplasticity and regeneration. To determine if circulating neurotrophic activity is altered by spinal cord injury, we measured T-3 weight drop to produce known levels of injury and in vitro neuronal models to determine the resultant trophic response. Anesthetized adult Wistar rats (250 g) underwent surgery to produce a mild or medium spinal lesion. Blood was collected 4 days after surgery from sham and injured rats and was kept at 4°C in aliquots at 70°C. Serum taken at day 0 prior to surgery served as a control. Control or trauma-derived serum was added to cultures of 6 day embryonic chick spinal cord (AHC) or 9 day sensory ganglia (DRG) which were maintained in vitro. The degree of resultant neuronal developmental was evaluated microscopically on coded cultures. Cell viability was determined by measuring mitochondrial dehydrogenases via MTT (2-[4,5-dimethylthiazol-2-yl]-5-(3-diphenyl formazan)bromide, Sigma cell growth determination kit). Sera obtained from normal rats established that minimal intra- and inter-animal variation occurred during the 28 day testing period. Our preliminary studies demonstrate that sera from lesioned animals contain factor(s) which increased the viability of the heterogeneous cell population. The degree of enhancement was dependent on the severity and time after injury. Maximum DRG and AHC viability was obtained with sera collected 4 days to 1 week after injury by day 28. The viability level was related to extent of injury. Sera obtained from animals with mild lesions were more supportive than those obtained from animals given a medium lesion. The effects of increase lesion-seeds survival are under evaluation with immunohistochemistry and quantitative microscopy. At present the factor(s) responsible are unknown. Supported by Alliant Community Trust Fund, Louisville, KY.

546.10


We have demonstrated an increase in septal cholinergic neuron survival and phenotypic differentiation in response to BDNF in vitro (Alderson, et al., 1990). We show here the effect of BDNF in vivo in the cholinergic neuronal population from the cell death associated with transection of the fimbria-fornix pathway.

The fimbria-fornix was transected unilaterally by knife cut out in adult (175-225 gm) Sprague-Dawley female rats. A cannula was implanted for delivery of BDNF into the caudal pole of the septum. BDNF was delivered continuously at rate of 12 μg/hr for two weeks via an osmotic pump. The ability of BDNF to increase cell survival was evaluated on the basis of cell counts in 30 μm coronal sections stained for choline acetyltransferase (ChAT) or low affinity NGF receptors (LNGFR). Cells were counted in lesioned and unlesioned sections taken at 180 μm intervals through the rostral-caudal extent of the medial septal nucleus. In control animals a 64% decrease in the number of ChAT and a 45% decrease in NGF receptors was observed on the lesioned side of the lesion compared to the intact side. In contrast only 37% of ChAT- and 21% of LNGFR-positive neurons were lost when BDNF was delivered into the septum. These results substantiate our previous in vivo findings and suggest a role for BDNF in rescuing cholinergic neurons following deafferentation injury or disease.
646.11 EFFECT OF INTRAVENTRICULAR BDNF ADMINISTRATION ON HIPPOCAM- PAL BDNF and mRNAs in the Rat MUSCLE DERIVED RATS WITH PARTIAL SEPTO HIPPOCAMPAL LESIONS. J.L. Yeniero, B. Knösel. K.D. Beck and F. Heff. Andrus Gerontology Center, University of Southern California. Los Angeles, CA 90009-1019.

In situ hybridization was used to evaluate changes of mRNA expression of BDNF and its trkB receptor after daily intraventricular administration of BDNF and at intervals after partial fimbrial transection. 24 and 48 h after four lesion, expression of BDNF was decreased in the pyramidal cell layer of Ammon's horn, this effect being most prominent in CA2, CA3 and CA4. In contrast, expression of BDNF mRHa in the granule cell layer of the dentate gyrus was enhanced at these time points. BDNF administration not only completely abolished the lesion-induced increase of BDNF mRNA in the dentate gyrus, but also increased the pyramidal cell layer of the dorsal hippocampus expression as compared to controls. No effect of BDNF administration was seen on the lesion-induced decrease in the different fields of Ammon's horn. Neither fimbrial transection nor BDNF administration affected trkA mRNA expression after 24 or 48 h. Emulsion in situ hybridization analysis was in progress to assess whether, at the level of individual cells, BDNF and trkA share common patterns of mRNA expression. Our data suggest that injury of septal inputs results in sub-region specific changes of hippocampal BDNF mRNA expression, whereas trkA expression does not seem to be affected.

The experiments with exogenously administered BDNF suggest that BDNF can specifically alter its own expression in the dentate gyrus.

646.13 TCF-1 REGULATION OF HIPPOCAMPAL mRNAs FOLLOWING ENTORHINAL CORTEX LESION. T.E. Morgan, N.J. Laping, N.R. Nichols C.S. Young-Chan B.W. Bernstein*, and C.E. Finch. Andrus Gerontology Center, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90009-1099.

This study examines the role of transforming growth factor-β (TCF-1) in regulating glial fibrillary acidic protein (GFAP) and sulfated glycolipids (SGLP-2) mRNAs during lesion-induced, synaptic reorganization. GFAP, a marker for reactive astrocytes, and SGLP-2, with a positive role in membrane transport, increase in the deafferented hippocampal region after unilateral entorhinal cortex lesion (ECL). TCF-1 is a multifunctional peptide that plays an important role in peripheral wound healing. Results of RNA titration indicate that TCF-1 mRNA increases 5-fold at day 4 after 7 day ECL. In situ hybridization indicates that TCF-1 mRNA is localized to the brain regions that show elevated microglial reactivity (OX-42 immunoreactivity) after ECL. To assess TCF-1 contribution to lesion-induced synaptic reorganization, a cannula was implanted into the lateral ventricle of adult male rats on the same day as ECL. TCF-1 100 ng or vehicle was infused 24 hours before sacrifice; rats were sacrificed 2 or 4 days after ECL. Two days after ECL, the GFAP and SGLP-2 levels were unaffected by TCF-1 infusion. However, preliminary results show the expected elevation in GFAP and SGLP-2 mRNAs at 4 days after ECL was prevented by TCF-1 infusion. Thus, TCF-1 may regulate astrocytic reactivity and other events involving membrane transport during synaptic reorganization. Supported by NRS A AC-0598 (TEM), NRSA AC-0582 (NHL) and AG-0709 (CEF).


Two motorneurotrophic factors (MNNTF1 & MNNTF2), isolated from the perineurial muscle of 3-week old rats and shown to promote the growth of motoneurons in vitro, were also isolated from muscle and in vivo after anoxia, were tested for their efficacy on axotomized neurons in the facial region. The facial nerve of 10-day old rats was transected unilaterally distal to its exit from the stylomandibular foramen under hypothermia. MNNTF1 and MNNTF2, separated by Fcass gel electrophoresis and migrated at 35 and 75 kDa, respectively, were cut out from the Fcass gel, minced into small pieces and placed between the two nerve stumps. For controls, either blank or Fcass gel devoid of neurotrophic activity 1 and 2 wk postanoxia, rats were perfused with 4% paraformaldehyde in PBS, and brains were perfused. Microscopic cell numbers in the motor nuclei of the facial nerve were counted on cresyl violet stained sections. Each experiment was repeated 20 times. While no significant differences were observed in the number of motorneurons on the side of the lesion, a significant difference was observed on the contralateral side, which was numbered to determine the recovery. Results indicated MNNTF1 and MNNTF2 were only effective in reducing the severity of chromatolysis and increased the survival of motoneurons after anoxia. However, mean percentage survival with each factor was significantly greater than in motoneurons on the opposite sciatic nerve, and MNNTF1 and MNNTF2 in combination did not show synergistic action. It is concluded that responsiveness to neurotrophic activity of both MNNTF1 and MNNTF2, isolated from hindlimb muscle, is age-dependent, that MNNTF1 promotes survival of motoneurons of the spinal cord, but to cranial motoneurons as well.

646.16 SYNERGISTIC EFFECT OF MOTORNEUROTROPHIC FACTORS (MNNTF) 1 AND 2 ON SURVIVAL OF AXOTOMIZED MOTOENDOCULARIS OF SCiatric NERVE. R.M. Liong, N.W.A. Yu, L.S. Chen* and R. Ben- Nemt. Dept. of Anatomy, Univ. of Hong Kong, Hong Kong; Dept. of Cell Biology & Anatomical Sciences, CUNY Medical School, New York, USA and Dept. of Anatomy, Charing Cross & Westminster Medical School London, UK.

MNNTF1 (35kD) & MNNTF2 (23kD) were isolated from the perineurial muscle of 3-week-old rats by the "protein band-fishing" by cellular extract method. MNNTF1 and MNNTF2 are factors that could support the survival of anterior horn motoneurons. In vivo survival of axotomized motoneurons of sciatic nerve of 10-day-old rats were studied. The sciatic nerves were minced gel were placed near the site of the lesion for survival experiments for 2 weeks. The results revealed that the percentages of axotomized motoneuron survival compared to those of the respective contralateral side were 44.8 ± 7.5 for the control without factor, 76.5 ± 10.8 for the rescue experiments with MNNTF1, 86.8 ± 2.5 with MNNTF2 and 86.8 ± 2.5 for those with both MNNTF1 and MNNTF2. These results indicated that MNNTF1 and MNNTF2 can individually or jointly promote survival of axotomized motoneurons of the sciatic nerve, in vivo, both with statistical significance P<0.05, n=7; however, when both MNNTF1 and MNNTF2 are used concurrently they can provide a very significant synergistic effect on the survival of axotomized motoneurons, in vivo, with P<0.01, n=7. These results are consistent with previous in vitro evidence.

Our previous studies have shown that extracts prepared from embryonic (E16) chick brains can enhance the survival of developing spinal motoneurons during normal cell death or death induced by spinal deafferentation. The current experiments were designed to partially separate and characterize brain extract (BEX) survival activity, and to determine the effects of members of the NGF-like neurotrophin family on deafferentation-induced motoneuron cell death.

Thoracic spinal cord segments were removed from chick embryos (E2) creating a spinal gap and lumbar segments were assayed for motoneuron survival on E16. Daily treatment (E15-E15) with proteins from a 25%-75% ammonium sulfate fraction of BEX prevented motoneuron cell death induced by spinal deafferentation. BEX activity was partially separated and characterized with tandem gel exclusion chromatography (Sephadex G-50 and Bio-Gel P-60 ), preparative isoelectric focusing electrophoresis and PAGE. Preliminary results suggest that activity is confined to low molecular weight proteins (<20 Kd) with a pI of 8 (high activity) or 5 (low activity). Interestingly, while all members of the neurotrophin family tested (NGF, BDNF and NT-3) promote motoneuronal survival, only BDNF appears to support survival during normal cell death.

546.19
SEX STEROID AND CASTRATION EFFECTS ON THE REGENERATING EDL MUSCLE AFTER PERONEAL NERVE CRUSH. J. Komer, R. Barnardian, and F.L. Sweet. New York University Depart of Biology and Center for Neural Sciences. NY, NY 10003. Sex steroids have been shown to affect peripheral nerve regeneration in a muscle that has been typically characterized as essentially non-androgen sensitive, the rat extensor digitorum longus muscle (EDL). (Komer, J. and Barnardian, F.L. Soc. Neurosci., Nov. 1991.) We investigated the influence of sex steroids on regeneration of the crushed peroneal nerve from the perspective of muscle reinnervation and motor functional recovery. Sprague-Dawley male and female rats were divided into 3 groups: sham-nerve crushed; nerve-crushed; and castrated, nerve crushed. Another group of castrated male rats was nerve-crushed, implanted with testosterone propionate (TP)(20 μg, id.058",od.077") and given ORG 2766, an anti-androgenic steroid (5mg/kg). At 7 and 14 days, nerve conduits were implanted proximally to the crush site, delivering graded stimuli (0.1 V, 1 Hz) to determine motor unit recruitment and maximal twitch contraction amplitude. Super-maximal stimuli elicit isometric tetanic responses, tetanic fusion frequency, and an altered peak rate. PTP responses were similarly increased at fusion frequency and at 120 Hz, twitches after fatigue at 400Hz for 10s. The differences in expression between liver and brain suggest tissue-specific temporal expression of certain cytokines. Supported by MH38399.

OTHER FACTORS AND TROPHIC AGENTS: CYTOKINES

547.1
TEMPORAL EXPRESSION OF BRAIN CYTOKINES INDUCED BY LPS. S. De, R.M. Klein, Q. Wood and N.E. Berman. Dept. of Anatomy and Cell Biology and Pathology and Oncology, University of Kansas Medical Center, Kansas City, KS 66160.

Extensive tissue remodeling occurs in the brain during development and following injury. Many of the cellular events of tissue remodeling, including phagocytosis of cellular debris, are accomplished by brain macrophages and microglial cells. In culture, these cells produce cytokines in response to stimulation by bacterial lipopolysaccharides (LPS). To determine how these cells respond to LPS stimulation in vivo, we injected LPS intraperitoneally or directly into the brain of adult CD-1 mice and studied the mRNA levels for various cytokines (IL-1α, IL-1β, and TNF-α) in the brain and liver using Northern blot analysis. Following intraperitoneal injection of 10 μg LPS, tumor necrosis factor alpha (TNF-α) message was increased in the liver at 1 and 2 hr, decreased by 4 hr, but increased again significantly 4 days later, stayed high for up to 7 days, and declined to the basal level by day 8. TNF-α expression in the liver was stimulated at 2 hr, declined to 4 hr, and stayed low through day 8. Following LPS injection into the brain, changes in interleukin-1-alpha and beta (IL-1α and IL-1β) expression followed the same time course in brain and liver. Both were increased at 4 hr but declined by 1 day and stayed low until 8 days, showing no late second peak in expression levels. These results demonstrate a biphasic temporal response of TNF-α mRNA in brain following LPS stimulation. The differences in expression between liver and brain suggest tissue-specific temporal expression of certain cytokines. Supported by MH38399.

The cytokine interleukin-6 (IL-6) mediates a number of biological activities, including hepatic acute phase response, maturation of activated B-lymphocytes, and inflammation. In addition, IL-6 exerts specific effects in the central nervous system, such as activation of the hypothalamic-pituitary-adrenal (HPA)-axis, promotion of mesencphalic neuron survival, and the neuronal plasticity associated with neurotrophic factor production. Using reverse transcription followed by polymerase chain reaction we investigated the expression of IL-6 and its receptor mRNAs in rat brain during postnatal development. The results of this study demonstrate that IL-6 mRNA is expressed in several brain regions, including the bed nucleus of the stria terminalis, the arcuate nucleus of the hypothalamus, the hippocampus, the lateral septum, the ventral tegmental area, and the substantia innominata. The expression of IL-6 mRNA was highest during the first week of life, and declined thereafter. The expression of the IL-6 receptor mRNA was also highest during the first week of life, and declined thereafter. The expression of the IL-6 receptor mRNA was highest during the first week of life, and declined thereafter.
In order to study the effects of immune cytokines on cholinergic neurons of the basal forebrain, we have grown dissociated neurons. Cells plated at 2 X 10^6 cells/ml showed a 12-fold increase in ChAT activity over cells plated at 1/4 that density. In subsequent studies, therefore, density was carefully maintained at 1.5-2 X 10^6 cells/ml. ChAT activity increased steadily over the first week in culture; then it slowly declined.

Recombinant murine IFN-γ (Schering-Plough) produced a dose-dependent increase in ChAT activity in one-week-old cultures taken from embryonic day 16 (E16). At 50 U/ml this peaked, showing a 10-fold increase over control. Steri-statal ChAT activity measured in sister cultures was unaffected by IFN-γ. The effect of IFN-γ was dependent upon fetal age. Cultures taken from E18 rats showed a significant but truncated (1.7-fold) increase in ChAT. E19 cultures did not respond to IFN-γ even at 500 U/ml.

The effect of IFN-γ is partially reversed in the presence of an antibody to NGF (5%; Mike Coughlin, McMaster Univ.), suggesting that NGF or another neurotrophin mediates at least some part of IFN-γ activity.

Supported by the Charles and Johanna Busch Bequest.

Further, expression of NPY is highly influenced by cell-cell contact. Observations indicate that although norepinephrine and NPY are often similar to or identical with CNTF, suggesting that the factor also mediates neuronal phenotypic changes. (Supported by ONR and NIMH.)

Neurons are known to be responsive to a broad range of biologically active peptides. Considerable information has been accumulated describing peptide neurotrophic factors. At the same time, the physiological role of these factors has not been fully elucidated. We have examined the effects of several cytokines during acute inflammatory episodes. We have examined the effects of several cytokines during acute inflammatory episodes. We have examined the effects of several cytokines during acute inflammatory episodes. We have examined the effects of several cytokines during acute inflammatory episodes. We have examined the effects of several cytokines during acute inflammatory episodes.

Cholinergic differentiation factor (CDF), identical to leukemia inhibitory factor (LIF), induces cholinergic phenotype in normally adrenergic sympathetic neurons. LIF mRNA is also found in rat CNS. We tested whether LIF might play a role in the transmitter-specific development of rat mesencephalic neurons. Chronic treatment of primary cultures of embryonic (E15) ventral mesencephalon with LIF reduced the uptake activity for radioabeled dopamine (DA) to approx. 50% of control after 1 week in vitro. Immunocytochemical staining of the cultures for the activity of the cholinergic marker enzyme choline acetyltransferase (CHAT) was increased two-fold. The reciprocal effects on cholinergic and dopaminergic properties were not due to transdifferentiation of DA neurons. Selective elimination of DA cells by pretreatment with 10 μM MPP⁺ for 24 hrs abolished DA uptake of both, control and LIF treated, cultures but did not affect the LIF mediated CHAT activity increase. Our data support that LIF affects cholinergic and dopaminergic mesencephalic neurons by the same, albeit indirect mechanism, possibly reflecting a specific, receptor mediated effect of LIF on development and differentiation of astrocytes.


Recent analyses suggest that ciliary neurotrophic factor (CNTF) and a number of hematopoietic cytokines including interleukin-6 (IL-6) and leukemia inhibitory factor (LIF) appear to be distantly related, as are some of their receptor components. While LIF shares several common actions with IL-6 outside of the nervous system, it also elicits responses in some neuronal cells similar to those of CNTF. We have identified biologically relevant responses to CNTF in a sympathetic neuronal progenitor cell line (MAH), which also responds identically to LIF. Comparison of the tyrosine phosphorylations and gene activations induced by CNTF and LIF in MAH cells, as well as in other neuronal cell lines, reveals that they are indistinguishable and are also very similar to signalling events which characterize IL-6 and IL-6 responses in hematopoietic cells. We provide a basis for the overlapping actions of these related cytokines by demonstrating that the shared CNTF and LIF signalling pathways involve the IL-6 signal transducing receptor component gp130. Thus the receptor system for CNTF is unlike the IL-3 receptor kinase used by the nerve growth factor family of neurotrophic factors, but instead shares components with the receptor complexes for a subclass of hematopoietic cytokines.

NUTRITIONAL AND PRENATAL FACTORS

548.1 EFFECT OF PRE- AND/OR POSTNATAL CHOLINE SUPPLEMENTATION ON WORKING AND REFERENCE MEMORY (SPATIAL AND NONSPATIAL) AND OPEN FIELD BEHAVIOR IN THE RAT. R.C. Peet* and J.J. Johnston. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1Z4.

Research has shown that choline supplementation during development enhances spatial working and reference memory in adult rats (Meck et al., 1989). Cholinergic blockade studies reported memory impairment (Eilen et al., 1986) and diminished responses to novel cues as well as a disruption of normal search and exploratory behaviors (Whishaw & Tomin, 1987).

The present experiment was designed to assess the effect of dietary choline supplementation on spatial and non-spatial memory performance and open field behavior in the rat. Subjects received either no supplementation, supplementation prenatally to postnatal day 30, or supplementation from postnatal days 16 to 30. Weck battery consisted of an open field task, visual discrimination task, 17-arm radial arm maze and delayed-nonmatching-to-sample.

Significant group differences were found, as well as a significant group by sex interaction effect in the postnatal group. These findings suggest that dietary choline supplementation enhances several aspects of memory performance and that postnatal supplementation alone has a differential effect depending on sex.

548.2 SEX DIFFERENCES IN THE EFFECT OF PRENATAL CHOLINE TREATMENT ON SEPTAL CELL SIZE AND HIPPOCAMPAL NGF. R. Loy*, D. Heyer, J. Miller and M.D. Lindner. Canadianaig VASMC and Department of Neurology, University of Rochester, 435 E. Henrietta Road, Rochester, NY 14620.

Choline treatment restricted to embryonic days 12-17 (E12-17) is sufficient to produce long-lasting enhancement of spatial memory in male and female rats, although the effect in females is not as robust. We tested rats treated in utero from ED12-17 with choline chloride (300 mg/kg/day, p.o. to the dam) for changes in the size of NGF receptor-positive neurons. Basal forebrain NGF receptor-positive neurons increased in size from 70 sq microns on PD0 to more than 200 sq microns on PD30. Males tended to have larger NGF receptor-positive neurons than females, but choline increased cell size in both males and females. Cells were no longer increasing in size after PD30, and by PD90 only the males still exhibited a significant increase in cell size due to choline treatment. Choline treatment also increased levels of NGF from ED12-17 to PD90 only the males still exhibited a significant increase in cell size due to choline treatment. Choline treatment also increased levels of NGF from ED12-17 to PD90 only the males still exhibited a significant increase in cell size due to choline treatment. Choline treatment also increased levels of NGF from ED12-17 to PD90 only the males still exhibited a significant increase in cell size due to choline treatment. Choline treatment also increased levels of NGF from ED12-17 to PD90 only the males still exhibited a significant increase in cell size due to choline treatment. Choline treatment also increased levels of NGF from ED12-17 to PD90 only the males still exhibited a significant increase in cell size due to choline treatment. Choline treatment also increased levels of NGF from ED12-17 to PD90 only the males still exhibited a significant increase in cell size due to choline treatment. Choline treatment also increased levels of NGF from ED12-17 to PD90 only the males still exhibited a significant increase in cell size due to choline treatment.

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Prenatal exposure to ACh content and the turnover rate of ACh (TRAcH) in specific brain regions of maternal and weaned female rats (Dev. Brain Res. 57: 296, 1990; 64: 183, 1991). In order to determine whether abnormalities in cholinergic function persist into adulthood, the following experiment was performed. On gestational day 7, female Sprague-Dawley rats anesthetized with methoxyflurane were implanted with 14-day Alzet osmotic minipumps filled with water (W) or 5% (each sex) and the pups fostered to untreated dams. On postnatal day 90 (P90) and beginning 11 min after i.p. injection of saline or morphine (M), rats were administered 5.62 mg/kg Fluoxetine HC1 by gavage beginning day 2 of pregnancy and ending the day of birth. A control group received distilled water by gavage during gestation. We analyzed effects of prenatal malnutrition on central neurotransmitters were assessed in postnatal day 1-3, 5-7, 9, 11 and 22 days old rats. Malnourished rats (6%), males born to dams fed a 6% casein diet (25% control), showed an enhanced basal release of hippocampal serotonin(5HT) and dopamine (DA), norepinephrine (NE) and their acidic metabolites from incubated slices of 6/25 as compared with controls (25% control) group. The basal release of these neurotransmitters in 90 days old animals showed a similar pattern, however, was less significant. "These findings indicate a long-term effect of Fluoxetine HC1 was shown to cause bleeding episodes in 6/25 group. The basal release of these neurochemicals show no difference in KCl-induced neurochemical release between the two groups at both ages. (2) Tissue concentrations of SRIF, DA, NE, their precursors and metabolites from hippocampus, striatum, cortex and brain stem were similar in the two groups at each age. However, individual chemicals displayed ontogenic difference during postnatal development and varied in different brain regions. (3) by SDS-PAGE and Western immunoblotting, we found there was a difference in contents of striatal and hippocampal synaptophysin between 6/25 and 25% groups in 90 days old rats. We hypothesized that some molecular species that regulate neurotransmission are vulnerable to prenatal protein malnutrition. (HD-2539).
548.9


We have previously analyzed the effects of prenatal malnutrition and postnatal rehabilitation on pyramidal cells in CA1 in adult rats. In this study we used the same paradigm in 30, 90 and 220-day-old rats. 216 pyramidal cells were studied using an imaging system. We measured the somal size, linear dendritic extent in relation to strata: radiatum and lacunaeum molecular and the number of spines in three 50 micron segments corresponding to perforant path (PP), Schaffer collaterals (SP) and commissural fiber systems. We found significant reductions (p<0.05) in some cellular parameters in 30-day-old rats and most of these parameters in 220-day-old rats in malnourished animals. At 90 days, the spine length and apical dendritic diameters were significantly reduced (p<0.05). Prenatal malnutrition produces severe alterations in the apical dendrites where both PP and SP synapse in 30 and 220-days-old rats. (Supported by DGAPA IN202891, NIH HD-22539-04, HD-23338-03).

548.10


We examined the ability of freely-moving 15- and 30-day-old prenatally protein malnourished rats (designated 6/25) to support and maintain LTP of the perforant path/hippocampal dentate gyrus granule cell synapse. Tetanization in 6/25 resulted in a 37% enhancement of the EPSP slope measure at the 5 hr post-potentiation period. This level slowly decayed to 18% after 24 hrs. Enhanced synaptic activation, however, was restored into an enhanced cellular discharge. Population spike amplitudes from 6/25 animals declined continuously, dropping to 30% of baseline 24 hrs after potentiation. Thus, tetanization in the 6/25 group resulted in a net decrease in memtransmission efficacy, even after prolonged culture. In conclusion, population spike component. Well-nourished controls did show enhancement in this measure beginning 3 hrs after potentiation, reaching levels of 50-70% above baseline 24 hrs after potentiation. Since no significant age-related differences were observed for baseline measures of population EPSP slope or population spike amplitude, the differential response to tetanization seen in 6/25 animals does not appear to result from immature granule cell physiology. We suggest that the dietary insult may impact intrinsic or extrinsic neuronal systems modulating dentate granule cell excitability. The findings have important implications for the need for full maintenance of LTP, suggesting possible consequences for learning and memory function as a result of the animal's inability to properly receive, process or store information during the early post-weaning period. Supported by NIH/CHD Grant # HD-22539.

GLIA AND OTHER NON-NEURONAL CELLS V

549.1

REGULATION OF GLIA-DERIVED NEKIN IN PRIMARY SCHWANN CELLS. A. Bleuel and D. Monard*

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Glia-derived nekmin (GDN) is a 43 kDa neurite-promoting glycoprotein with serine protease inhibitory activity. In vivo experiments have shown that expression of GDN is dramatically induced in the sciatic nerve of mice following peripheral nerve injury (Meier et al., Nature 343, 548-550, 1990) and following hippocampal lesion caused by transient forebrain ischemia in the gerbil (Reffert et al., Neuroscience, in press).

In explant cultures of dorsal root ganglia, the GDN level is down-regulated and, in vivo, can be induced following lesion of the processes emerging from the explants. The GDN increase detected by immunocytochemistry is restricted to the Schwann cells distal to the site of injury, where nerve degeneration has taken place. Neuritophic factors, also known to be induced after lesion, have been tested for their ability to affect GDN expression in pure Schwann cell cultures. A significant increase of GDN mRNA and protein is seen after NGF treatment. NT3 and EGF remained inactive. Some neuropeptides also influence GDN synthesis. These results suggest that neuron-glial interaction, both by cell-cell contact and secretion of macromolecules, plays an important role in the regulation of GDN.

549.2

AGE-RELATED DIFFERENCES IN PROLIFERATIVE RESPONSES OF SCHWANN CELLS DURING WALLERIAN DEGENERATION. A. Korinayama*, K. Hasegawa and K. Suzuki, Dept. of Pathology, Univ. of North Carolina, Chapel Hill, NC 27599 and Dept. of Neurology, Yokohama City Univ. Sch. of Medicine, Yokohama, Japan.

Proliferative responses of Schwann cells during Wallerian degeneration were investigated in the mouse sciatic nerves after nerve transection at 3, 10 and 20 days, corresponding to the periods of early myelination, active myelination and postmyelination. As assayed by thymidine incorporation for the first 24 hrs in culture, Schwann cells from adult nerve proliferated rapidly within day 1 post-transsection and reached a peak at day 3. In the nerves from neonatal or suckling mice, however, division rate of Schwann cells declined after transsection, and was even less in the transected nerves than in the contralateral uninjured ones. The reduction in thymidine uptake by Schwann cells was more pronounced in nerves sectioned at postnatal day 3 than those sectioned at day 10. By contrast, fibroblasts divided rapidly following transsection regardless of age. These data suggest that in the degenerating nerves of young mice, their mitotic capacity of Schwann cells declined because of a loss of axonal mitogens and also the paucity of mitogens from myelin components, whereas proliferation of fibroblasts is stimulated by growth-promoting polypeptides common to any other tissues during wound repair.

549.3


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We have previously reported to the Society that a conditioning lesion to the sciatic nerve of adult rats facilitates the isolation of Schwann cells from the distal portion of the nerve several days later (Soc. Neurosci. Abstr. 17:932, 1991). It appears that the process of Wallerian degeneration causes both an increase in the number of Schwann cells in situ and makes it easier to extract the cells using conventional methods. We have exploited this observation further, and have found that the enzymatic treatment required to isolate cells from sciatic nerves can be much gentler than those required for intact nerve. This change in procedure produces a further reduction of the digestion phase from 18h to 4h. The cells from each nerve segment were counted and processed as previously described except for reduction of the digestion phase. The number of Schwann cells are removed from axons. However, in chickens, P0, as detected by 1E8, is expressed prior to myelin formation. As in rodent, most Schwann cells purified using the 1E8 monoclonal antibody to immunoselect P0-positive Schwann cells from embryonic 14(14)1 chick sciatic nerve. When cultured alone, but were responsive to mitogenic stimulation by neurons and peptidase common to any other tissues during wound repair.
Goldfish Schwann cells and optic nerve oligodendroglialike cells are distinct cell types.

M. Bastersmyer and C.A. Steuer, Faculty of Biology, University of Konstanz, Germany.

Oligodendroglialike cells isolated from the goldfish optic nerve share many properties with mammalian Schwann cells. In particular, like mammalian Schwann cells but unlike mammalian oligodendrocytes, the fish oligodendroglialike cells support the growth of regenerating axons (Bastersmyer et al., J. Neurosci. 11, 626-640, 1991). Therefore, we isolated fish Schwann cells and compared them to the oligodendroglialike cells (B.P.S.).

Fish gliial cells were obtained by explanting small pieces from the IX and X cranial nerves of adult goldfish onto laminin coated coverslips. Two morphologically and immunocytochemically distinct cell types emerged and multiplied in culture: long, bipolar Schwann cells and fibroblasts. Like goldfish oligodendroglialike cells, fish Schwann cells expressed the O4-antigen and the fish myelin proteins P1/2, which are related to mammalian P0. Both, Schwann cells and oligodendroglialike cells carried the HK1-epitope, NCAM and the E587-antigen, which is related to N-CAM and the E587-antigen, which is related to L1. (Vielmetter et al., J. Neurosci. 11, 3581-3593, 1991). Oligodendroglialike cells differed from fish Schwann cells in that they transiently expressed fish-GFAP. Only the Schwann cells but not the oligodendroglialike cells were immunoreactive for anti-NGF-receptor. Thus, despite substantial similarities, goldfish optic nerve oligodendroglialike cells and goldfish PNS Schwann cells are distinct cell types.


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We have detected mRNA for B-50 (GAP 43, pp46, F1, neuromodulin) in sciatic nerve tissue. In situ hybridisation demonstrated B-50 mRNA associated with Schwann cells in the distal nerve stump. The observation that Schwann cells are capable of producing B-50 mRNA was confirmed by Northern blot analysis of total RNA isolated from primary Schwann cell cultures. Taken together these data suggest that B-50 mRNA is transiently expressed in Schwann cells treated with 24 hr supernatants from cultures treated with 24 hr supernatants from regenerating nerve constituents which increase Schwann cell proliferation significantly; 12 hr, 4d and 8d supernatants produced increased Schwann cell proliferation significantly; 12 hr supernatants increased Schwann cell proliferation significantly; 12 hr, 4d and 8d supernatants produced smaller increases. The nature and origin of regenerating nerve constituents which increase Schwann cell proliferation and laminin production in our cultures is being investigated.

The expression of C-Jun protein in Schwann cells depends on their environment.


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The Schwann cell, in contrast to glia derived from the adult CNS, is considered a permissive substrate for axonal regeneration. CNS myelin proteins are abundant, and axons grow over them. CNS glial cells have an axon inside a peripheral nerve graft implanted into the CNS, although such regeneration growth of regenerating axons (Bastersmyer et al., J. Neurosci. 11, 626-640, 1991). Therefore, we isolated fish Schwann cells and compared them to the oligodendroglialike cells (B.P.S.).

Fish gliial cells were obtained by explanting small pieces from the IX and X cranial nerves of adult goldfish onto laminin coated coverslips. Two morphologically and immunocytochemically distinct cell types emerged and multiplied in culture: long, bipolar Schwann cells and fibroblasts. Like goldfish oligodendroglialike cells, fish Schwann cells expressed the O4-antigen and the fish myelin proteins P1/2, which are related to mammalian P0. Both, Schwann cells and oligodendroglialike cells carried the HK1-epitope, NCAM and the E587-antigen, which is related to N-CAM and the E587-antigen, which is related to L1. (Vielmetter et al., J. Neurosci. 11, 3581-3593, 1991). Oligodendroglialike cells differed from fish Schwann cells in that they transiently expressed fish-GFAP. Only the Schwann cells but not the oligodendroglialike cells were immunoreactive for anti-NGF-receptor. Thus, despite substantial similarities, goldfish optic nerve oligodendroglialike cells and goldfish PNS Schwann cells are distinct cell types.

The expression of c-Jun protein in Schwann cells depends on their environment. E. Vaudano & C. de Haan.

The expression of c-Jun protein in Schwann cells depends on their environment. E. Vaudano & C. de Haan.

The role of glucocorticoids in Schwann cell proliferation. T. Neuburger and G.H. Devries, Dept. of Biochemistry, Medical College of Virginia, Richmond Va. 23298.

The asolectin enriched fraction (AEF) contained potent mitogens, which in the presence of serum induces Schwann cell proliferation. In serum free media, the AEF also induces Schwann cell proliferation but only if hydrocortisone is included in the media. Schwann cells grown in defined media containing hydrocortisone demonstrated a 5 to 10 fold greater response to AEF stimulation compared to Schwann cells grown in defined media alone. Moreover, the AEF stimulated goldfish oligodendroglialike cells in mediating the response of Schwann cells to the AEF associated mitogens was further investigated using two separate approaches, a) chemical extraction of steroids from serum and b) inhibition of the glucocorticoid receptor.

The addition of AEF to Schwann cells in serum containing media resulted in a 50% reduction in cell proliferation as compared to the AEF added to serum free media. The effect was completely reversed by the removal of RU-486 from serum media 24 hours prior to the addition of AEF. Since the rate of 'H-thymidine by AEF stimulated Schwann cells was not affected by pretreatment with increasing concentrations of RU-486, RU-486 did not appear to adversely affect Schwann cell viability. Finally, increasing concentrations of RU-486 added to serum containing media resulted in morphological changes. Schwann cells grown in serum containing media had short, thick processes and formed large aggregates of cells. In contrast, in serum free media and serum containing media in which 100% of the Schwann cells had long, thin processes and were generally observed as isolated cells. This data suggests that the proliferative response of Schwann cells to the AEF in both serum containing and serum free media is dependent on the availability of glucocorticoids in the media.

This work was supported by NS 15408 and HL-07110-15.

The effects of nerve segment supernatants on cultured Schwann cell proliferation and laminin production. O. Wang, G.L. Stoner, and H. Webster.

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Mouse sciatic nerves were transected and 3 hr, 16d later, proximal segments were removed and homogenized. Supernatants from these segments were added to normal sciatic nerves were added to Schwann cells maintained in DMEM+15% FCS. After 5d, Schwann cells were solubilized and the protein content was measured using a Bradford protein assay. Samples containing the same amounts of protein were applied to microtiter plates and the laminin content was determined by ELISA. Samples derived from cultures treated with 24 hr supernatants contained significantly higher levels of laminin than controls or other intervals. Increased surface and cytoplasmic a-laminin immunoreactivity was found in Schwann cells treated with 24 hr supernatants. Schwann cell proliferation was compared in supernatant-treated cultures by using a 24 hr ELISA. The 24 hr supernatants increased Schwann cell proliferation significantly; 12 hr, 4d and 8d supernatants produced smaller increases. The nature and origin of regenerating nerve constituents which increase Schwann cell proliferation and laminin production in our cultures is being investigated.

The developmental regulation of growth factor receptor expression in the rat sciatic nerve: correlation with the period of Schwann cell proliferation. J.B. Davis.

The development and myelination of peripheral axons, Schwann cells go through consecutive periods of proliferation, growth and differentiation. Schwann cell proliferation occurs prior to myelin gene expression.

The control of Schwann cell proliferation during the developmental process may be achieved by a) a regulatory role in the growth factor, b) control of Schwann cell receptor expression, or c) a dominant inhibitory signal. In order to test a & b, we have studied the expression levels, in sciatic nerves, of PDGF and FGF, and their receptors, over the period of P0-P14 period, by means of northern analysis.

There is a rapid decline in FGF and PDGF-B receptor mRNA expression over PO-P3, which correlates with the rapid decrease in Schwann cell proliferation rate over the same period. In contrast, the expression of the mRNAs for the growth factors remains constant. PDGF-B chain mRNA is absent, whilst FGF expression is retained in the adult. These data are consistent with the theory that the growth factor receptor expression might control Schwann cell proliferation during development.

This work was supported by an EMBO fellowship.
Neuroepithelial and mesodermal components associated with the Vth nerve were labeled by MO-1 monoclonal antibody (MoAb) and the application of lipophilic fluorescent dyes in *Squalus acanthias* embryos. Motoneurons originated in rhombomere 6 and by stage 23 axons extended to the spinal cord to form the spinal motor nucleus. At stage 24, Vth nerve growth cones were observed to contact two separate epidermal-like rudiments of mesodermal origin. A distinct hypothalamic-mesencephalic tract was located medial to the Vth ganglion and a mandibular component, Platt's muscle E, lay slightly more rostral. Each precerebellar muscle rudiment received a separate Vth nerve branch. Confocal microscopy showed that some dye labeled abducens motoneurons in contact with these mesodermal targets retained ventricular attachment in the neuroepithelium. Between stages 25-27 the two pre-muscle masses maintained their epithelial organization while extending forward onto the globe where, by stage 29, they merged together. These observations demonstrate an autonomous development of two separate mesodermal derivatives that each receive a Vth nerve branch. Some motoncyes are displaced rostrally before fusing to form a common lateral rectus muscle. A caudally directed Vth nerve branch associated with the most caudal Vth nerve rootlet was present at stage 25-26. Dye applied to the caudal branch labeled peripheral ganglion-like cells and showed entry of fibers into the neuroepithelium, but revealed no motoneurons. These data suggest that the formation of a series of eccentric neural crest-derived ganglia associated with the cranial nerves.


It has been proposed that there is a relationship between motoneuronal size and the size of its peripheral field, i.e. motor unit (MU) size. In the rat, by postnatal day 21 (P21), synaptic growth and myogenesis is complete for the diaphragm and medial gastrocnemius (MG) muscles. We hypothesize that subsequent growth of muscle fibers is paralleled by growth of phrenic and lumbar motoneurons (MNs). We obtained estimates of muscle fiber volume by measuring optimal fiber length and fiber cross-sectional area (CSA). Phrenic and lumbar MNs were retrogradely labeled with tetramethylrhodamine dextran via intravascular injection. "Optical slices" of labeled MNs were viewed using a Bio-Rad Lacaluma Confocal microscope. In both muscles at D21, type I and II muscle fibers were comparable in size, while type II fibers were larger in both adult muscles. Adult MG fibers were significantly larger than DIA fibers. Since innervation ratios are constant by D21, postnatal changes in fiber volume reflect MU growth. Phrenic MN somal volumes doubled between stages D10 and 10 weeks (adult), while lumbar MNs nearly tripled in size. At each age lumbar MNs were larger than phrenic MNs. This is consistent with the growth of the MU size in the two muscles, although MU growth was not in direct proportion to changes in somal volume. A bi-modal distribution of somal volume was evident in adult MG MNs, but less so in the phrenic pool. We speculate that the bi-modal MN volume distributions reflect differences between slow and fast twitch MUs. Bi-modal distributions were less obvious at D21, which may reflect the absence of homogeneity in fiber CSA between different muscle types at that age. Supported by NHI grants HL34817 and HL37680.

**550.4** A motor neuron-specific epitope and the low-affinity nerve growth factor receptor display reciprocal patterns of expression during development, axotomy and regeneration. A.Y. Chiu,* E.W. Chen and S. Loera, Division of Neuroscience, City of Hope, Duarte, CA 91010.

Embryonic motor neurons express the low-affinity Nerve Growth Factor Receptor (NGFr), but in postnatal life, they lose NGFr immunoreactivity, and acquire a motor neuron-specific epitope that is recognized by the monoclonal antibody, MO-1. We examined the effect of nerve injury on these two developmentally regulated markers in two populations of somatic motor neurons. Unilateral ligation or crushing of the sciatic nerve resulted in loss of MO-1 and a concomitant rise in NGFr immunoreactivity within motor neurons in lumbar levels of the spinal cord. Transection of the hypoglossal nerve, a pure motor nerve, resulted in a similar loss of MO-1 binding and a concomitant rise in NGFr immunoreactivity in neurons within the ipsilateral hypoglossal motor nucleus. These changes, detectable within 5 days following nerve injury, are reversed with reinnervation, but persist if reinnervation is prevented by chronic axotomy. Thus, regulation of the expression of NGFr and the MO-1 epitope appears to be dependent upon interactions between motor neurons and target muscles. These observations are also consistent with the idea that regenerating neurons may revert to a developmentally immature state; in the case of motor neurons, this state is characterized by the presence of NGFr and the absence of the MO-1 epitope.

**550.5** THE ROLE OF EXTRACELLULAR CALCIUM AND CALCIUM CHANNELS IN ACTIVITY DEPENDENT INTRACELLULAR CALCIUM CHANGES IN EMBRYONIC CHICK MOTONEURONS. Michael O'Donovan* and Stephen Bass. Section of Neurobiology and Behavior, Cornell Univ., Ithaca, N.Y. 14853 and Bodega Marine Laboratory, Univ. California, Bodega Bay, CA. 94923.

Sexually mature males and females of the plainfin midshipman (*Porichthys notatus*) vocalize by the simultaneous contraction of sonic muscles attached to the lateral walls of the swimbladder (review: TINS 15:139). Previous studies have demonstrated that each muscle is innervated ipsilaterally by motoneurons located in caudal brainstem sonic motor nuclei. Biocytin was used as a retrograde tracer to identify paired, midline sonic motor nuclei in specimens ranging from 83.5-25.5 cm total length (~14-40 days post-fertilization). Following ipsilateral applications of biocytin to the sonic muscle, there is a Coli-like filling of motoneurons; label is bilateral with increasing survival times. During these stages, myofibers differentiate and sonic motor axons form "typical" motorneuronal junctions (cf. Brain Res. 251:312). Surprisingly, sonic motoneurons at these stages do not express choline acetyltransferase (ChAT), the rate limiting enzyme in the synthesis of acetylcholine. In contrast, other nearby motor nuclei are ChAT-positive, as are sonic motoneurons in sexually mature adults (AB8 monoclonal antibody; cf. J Neurosci. 27:7876). Therefore, the following conclusions can be drawn: (1) acetylcholine synthesis is not required for early events in motor neuron and muscle fiber differentiation in this vocal pathway, and (2) there is late onset of ChAT expression, perhaps linked to sexual differentiation of the vocal motor phenotype. Supported by NSF, Cornell University Biotechnology Program and New York State Hatch Act.

The small Brazilian opossum Monodelphis domestica is born more immunologically inert than placental mammals. At birth, the hindlimbs (HL) are little more developed than embryonic buds that do not move independently of the trunk, and HL muscles are not all individualized (Astrow & Thompson, Soc. Neurosci. Abst. 153.5, 1989). It is possible to observe perinatal development of HL motility and the coordination between them and the forelimbs (Cassidy et al., Soc. Neurosci. Abst. 377.6, 1991). Furthermore, supraspinal centers have not reached the lumbosacral enlargement (LS) of the cord at birth (Wang et al., Soc. Neurosci. Abst. 377.4, 1991). In view of such immaturity of the newborn opossum’s HL, we sought to verify if the connections between the two enlargements of the spinal cord, which contribute greatly to interlimb coordination and are more precocious than most projections descending from the brain, are established at birth. We have used DI and WGA-HRP as retrograde markers to study those connections. At day 1, projections to LS originate mainly from the ventral horn of the brachial enlargement, presumably from presumptive laminae VII-VIII, and, to a lesser extent, from presumptive laminae V-VI. Projections from these laminae increase after birth and those from the more dorsal laminae (Cabana & Parent, Soc. Neurosci. Abst. 282.2, 1989) are added. A proportion of propriospinal connections is thus established well before any behavioral evidence for them. Supported by NSERC.


Intraneural or perineural phenol injection is an accepted procedure used to produce a temporary but long lasting peripheral motor nerve block in plastic muscle. The purpose of this study was to evaluate the morphologic and functional effects of phenol application on peripheral nerves and to establish the degree to which sensory and motor recovery occur after the nerve block. A 5% aqueous solution of phenol was applied to the sciatic nerve of the rat by intraneural injection using a 30-gauge hamilton syringe or by bathing the entire nerve with the solution. Phenol was injected into the nerve or applied extraneurally until a complete motor block was produced, i.e., no detectable nerve conduction could be elicited with stimulation of the lower limb muscles. At 2 days after phenol application, production of the sciatic nerve and soleus (SOL) and tibialis anterior (TA) muscles were removed. At each time point, muscle contraction could not be elicited with stimulation of the sciatic nerve proximal to the site of phenol application. Phenol produced extensive degeneration of the axons and demyelination of the nerve fibers resulting in denervation of the SOL and TA muscles. At 2 days there was no significant atrophy in the SOL or TA. At 1 wk, the weights of the SOL and TA were 66 and 82% of control values, respectively. At 2 wks, the weights of the SOL and TA were 51 and 50% of control values, respectively. In general, the slow fibers atrophy more rapidly than the fast fibers. Phenol appears to produce a motor block by causing axonal degeneration without damaging the endoneurial tube. The degree to which axons reinnervate the appropriate muscle remains to be determined.


During the late prenatal period, fetal rats exhibit spontaneous motor activity that is characterized by synchronous movement, sequential organization, and temporal patterning (bouquet structure and cyclicity). The prenatal emergence of motor organization also appears to be evident in the expression of behavioral sets: discrete periods of time during which patterns of behavior that are similar both spatially and temporally take place. In the present study, fetal rats were directly observed on day 20 of gestation during 60-min sessions to characterize behavioral sets defined by spatial (part of the body) or temporal (intervals between successive movements) criteria. Motor activity comprising motoric elements (head, forelimbs) tended to alternate with periods predominated by caudal elements (rearrangements). Studies employing Monte Carlo techniques indicated that the duration of spatially-defined movement sets was longer than expected, whereas the temporal component was randomly ordered. Sets defined by temporal criteria (variability of inter-movement intervals) also persisted longer than expected by chance. There was no evidence for the presence of behavioral sets characterized by periods of high or low interval variability which were poorly associated. However, a single 20 μl infusion of milk into the lateral ventricle of the fetal rat increased the duration of rostral-caudal sets and promoted synchronization of spatial and temporal variables. The independence and stimulus-evoked coupling of these behavioral variables in fetal rats suggests that behavioral state organization may result from self-organization and not from the maturation of central regulatory mechanisms during the prenatal period. This research is supported by Grant HD 25231 to WPS and SRR.

550.11 ELECTRICAL ACTIVATION AND INHIBITION OF RESPIRATION IN VITRO. D. Hamada*, E. Garcia-Rill and R.D. Skinner, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Previous research has shown that electrical stimulation of the caudal ventrolateral pons and medulla in decerebrate cats that the most important peculiarity of their afferent control are common to the corresponding central pattern generators (CPGs). A concept of universal spinal motor control system, which is capable of controlling movements of posture and locomotor patterns and temporal patterning (bouquet structure and cyclicity), the present study was undertaken to determine the effect of electrical stimulation delivered to the medulla, the pons and the combination of both so some of the functional interactions within the spinal segments could be revealed. 25 rats aged 0-2 days were used in this study. Stimulation required to achieve respiration consisted of low frequency pulses (0.2-0.4 Hz) and the most effective area was the ventromedial medulla. In 15 of 19 cases, stimulation at 0.2-0.3 Hz activated respiration after the same frequency using low amplitude current (25-313.5 μA at 0.2 Hz, 27-138 μA at 0.3 Hz). In 7 out of these 15 cases, respiration followed stimulation pulses. Simultaneous stimulation of the ventromedial medulla inhibited the respiratory rhythm generated by the mediatory respiration generator (Hirai et al., 1989).

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This research is supported by Grant HD 25231 to WPS and SRR.


It was shown in experiments on scratching and locomotor reflexes in decerebrate cats that the neuronal control mechanisms that they activate are common to the corresponding central pattern generators (CPGs). A concept of universal spinal motor control system, which is capable of controlling movements of posture and locomotor patterns and temporal patterning (bouquet structure and cyclicity), the present study was undertaken to determine the effect of electrical stimulation delivered to the medulla, the pons and the combination of both so some of the functional interactions within the spinal segments could be revealed. 25 rats aged 0-2 days were used in this study. Stimulation required to achieve respiration consisted of low frequency pulses (0.2-0.4 Hz) and the most effective area was the ventromedial medulla. In 15 of 19 cases, stimulation at 0.2-0.3 Hz activated respiration after the same frequency using low amplitude current (25-313.5 μA at 0.2 Hz, 27-138 μA at 0.3 Hz). In 7 out of these 15 cases, respiration followed stimulation pulses. Simultaneous stimulation of the ventromedial medulla inhibited the respiratory rhythm generated by the mediatory respiration generator (Hirai et al., 1989).

This research is supported by Grant HD 25231 to WPS and SRR.
Visual Development: Striate Cortex


Thalamocortical afferents in layer 4 of the primary visual cortex are segregated according to eye of origin into alternating patches that represent the system of ocular dominance (OD) columns. The activity-dependent formation of OD columns during development and its perturbation by visual deprivation during the critical period has been thoroughly studied at the level of the thalamic afferents. However, it is not known whether this organization of the postsynaptic terminals is accompanied by a remodeling of their postsynaptic target cells in layer 4. In the present study we made in vivo injections of the fluorescent tracer DiI into layer A of cat LGN to label anterogradely thalamocortical afferents of one eye only. By intracellular injections of Lucifer Yellow in slice preparations we were able to visualize simultaneously the morphology of cells in layer 4 and the termination pattern of the thalamic afferents. A quantitative analysis of the dendritic branching revealed that spiny stellate cells with their somata in the middle of OD columns as well as in the middle of layer 4 had nearly radially symmetrical dendritic fields. Cells near the upper or the lower borders of the thalamic afferents showed strong asymmetries in their dendritic fields; their dendrites were for the most part confined to the afferent receptive zone. Finally, the dendrites of cells near the border between two OD columns showed a tendency to avoid crossing the OD border. We also examined monocularly deprived animals and found that cells in deprived visual cortex also respected the OD borders. In contrast, cells in monocularly deprived animals and found that cells in deprived visual cortex also respected the upper and lower borders of the thalamic input zone. Likewise, cells in the columns of the open eye respected the OD borders. In contrast, cells in the deprived columns extended their dendrites into neighboring columns. These results indicate that the dendritic fields of cortical neurons are shaped by patterns of afferents input. Thus, the segregation of thalamic afferents into OD columns during normal development and its perturbation by visual deprivation plays a significant role in defining the structure of cortical neurons.


Our aim was to investigate the early postnatal changes in the organization of the area 17-18 association projection.

We injected neuroanatomical tracers in the visual cortex of 10-30 day-old kittens. In some, the retrograde tracer diamidino yellow (DY) was injected in area 18 or the white matter below it, to label all bodies in area 17. In others, the axons of association cells in area 17 were visualized with anterogradely transported carbocyanine dye, DiI and DiA.

Our results with DiI and DiA reveal that axons from area 17 have reached area 18 at birth. The majority of these fibres project highly divergently and terminate in deep layers; a few penetrate the superficial layers at regions topographically related to the position of the injection. As the pathway develops, the characteristic projection to the superficial layers becomes denser, while the exuberant projection to the deep layers is reduced. Results with DiI confirm these observations: injections involving the deep layers of area 18 and the underlying white matter produce more widespread labeling of area 17 than those restricted to superficial layers.

551.5 Interhemispheric Connections in Newborn Galagos (Galago crassicaudatus) and Owl Monkeys (Aotus trivirgatus). P. D. Beck and J. H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Interhemispheric connections of newborn primates were studied by injecting HRP and WGA-HRP at multiple sites in dorsolateral cortex of one cerebral hemisphere of three galagos and one owl monkey within the first postnatal week. Brains were cut in the coronal plane, or cortex was removed from the rest of the brain, sectioned, and cut parallel to the surface to aid reconstructing surface-view patterns. In both primates, the total pattern of callosal connections was remarkably adult-like. In all regions of cortex, labeled neurons and processes were unevenly distributed, and there was no clear evidence of excessive exuberance. Callosal connections were concentrated along the 18/17 border in both primates, and they extended only a short distance into area 17 in owl monkeys. In galagos, area 17 was densely connected to the other hemisphere, and connections tended to overlap the C CObls. In galagos, callosal connections were sparse in CO dense portions of S-I and dense in CO light portions. Such compartmentalization has not yet been reported in adult galagos, but it may be a common feature of S-I in mammals. (Supported by NEI EYO2686).


Our aim is to investigate factors that influence the development of the projection from the lateral geniculate nucleus (LGN) to the visual cortex using an in vitro organotypic culture system. Explants of occipital cortex and LGN from prenatal and neonatal mice were cultured on collagen-treated filters suspended in a chemically defined serum-free medium. Axons growing from geniculate neurons were labeled with the carbocyanine dye, DiI.

When the LGN from embryonic day 16 (E16) mice was cultured alone, large fiber tracts developed within it but appeared unable to emerge onto the collagen filter. However, the presence of the occipital cortex, or the use of medium conditioned by the occipital cortex, promoted the outgrowth of axons onto the filter.

These results suggest that the occipital cortex releases a diffusible factor that induces outgrowth from the LGN, although it is not necessary for growth within the nucleus. It is possible that this substance plays a role in directing geniculocortical axons towards the developing cortex in vivo.


A previous study using DiI to label corpus callosum (CC) connections in rat revealed a substantial number of transitory CC axons extending throughout visual cortex that gradually disappeared during the first postnatal month (Elberger, Soc. Neurosci. Abstr. 1991). These axons had large axon swellings and terminal dendritic swellings in all cortical layers; the swellings were hypothesized to be synapses. To evaluate this hypothesis in neonatal rats, biotinylated dextran amine was injected into the visual cortex of one hemisphere to anterogradely label CC axons. The rats were sacrificed during postnatal week 2 and coronal sections were histochromically processed (Wewnman et al., J. Neurosci. Meth. 41:239, 1992). In vivo organotypic co-culture system. Explants of cortex from mice were cultured on collagen-recoated filters and the formation of synapses by the abundant transitory CC axons extending throughout visual cortex provides an extensive opportunity for the CC to influence the development of visual cortex. Supported by NIH Grants (DK-47155) and State of Tenn. Neuroscience Center of Excellence (SLO).

551.6 Morphological Differentiation of Layer 5 Projection Neurons from the Immature Rat Visual Cortex Studied in Vitro. Ekkehard M. Kasper, Joseph Lübke and Colin Blakemore. University Laboratory of Physiology and Dept. Human Anatomy, Oxford University, Parks Road, Oxford, U.K.

Recent studies have demonstrated that projection neurons in layer 5 (L5) of the adult rat visual cortex differ in their intrinsic morphological and physiological characteristics which are correlated with their respective axonal projection target (Kasper et al., 1991). Corticocortical neurons have a prominent apical dendrite extending into layer 1 where it forms an extensive arborization. Interhemispherically projecting neurons possess a slender apical dendrite which tapers below layer 1 and does not arborize in form of an extensive terminal tuft.

To find out how early the two characteristic morphological types can be distinguished in cortical development, we have now studied L5 neurons in a fixed brain-slice preparation of late prenatal and early postnatal cortex (E17, E19, P1 - P5). Brains were perfused with a various postnatal rats and coronal sections were prepared. Individual cortical neurons were intracellularly injected with Lucifer Yellow, subsequently photoconverted, and drawn according to Ruhl and Lübke (1988).

At E17 and E19, no morphological differences were observed between L5 neurons. However, at P5 corticocortical and interhemispheral neurons had aquired their distinct morphology.
551.7 LOCALIZATION OF NEURONS DISTINGUISHABLY LABELLED AFTER INJECTIONS OF 125I-NGF INTO THE POSTERIOR CORTEX OF RATS DURING POSTNATAL DEVELOPMENT. 


We studied the distribution of neurons transporting labeled NGF, injected in the rat occipital cortex at various postnatal ages. 125I-labeled NGF (0.5-1 ul) has been injected into the posterior occipital cortex of rats at the postnatal age P14. 125I-labeled NGF was isolated by the chloroform T method (protein concentration=5 ng/ul; specific activity=5 x 10^7 cpm/ug). The animals were perfused (under chloral hydrate anesthesia) 24 hours later. After an exposure time of 3 weeks, the autoradio grams were developed and the slices counterstained with Cresyl Violet. The results showed that NGF was taken up and retrogradely transported by forebrain neurons at all the postnatal ages investigated. After injection of NGF at P14 and P20 labeled cells were found within the visual cortex ipsilateral to the injection site. These cells were not found in adulthood (3 months). In conclusion, the present results confirm earlier findings in forebrain neurons projecting to the visual cortex (M.Siebler and M.Schend, Brain Res. 300:33-39,1984) and further suggest that in cortical cells, the expression of receptors for NGF or related compounds could be modulated during postnatal development.

551.9 DEVELOPMENT OF BINOCULAR VISUAL FUNCTIONS IN MONKEYS. Rick J. Brown, James R. Wilson, Yvette P. Vieira, Jamie L. Green and Ronald G. Booth*. Dept. of Psychology, Ophthalmology, Anatomy & Cell Biology, and Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

A number of characteristics of binocular vision are immature at birth and develop concurrently during postnatal development. These functions are often disrupted by visual deprivation rearing, but few norms are available regarding the exact sequence of emergence during normal development. Thus, it is impossible to differentiate deprivation effects that involve failure to develop from effects that involve deterioration. We are addressing this issue by using a combination of cross-sectional and longitudinal methods to track normal development of several binocular functions in infant rhesus (Macaca mulatta) monkeys. Our methodologies used for these assessments include visually evoked potentials (VEP), optokinetic nystagmus (OKN), preferential looking (PL), and corneal reflex photography. During the first few weeks after birth, ocular alignment changes from intermittent exotropia to predominantly orthotropic; stereoscopic sensitivity to horizontal disparity emerges, and there is a conversion of an immature motion response from a retinotopic to predominantly striatotopic; the receptive fields become larger, and ocular dominance columns become more mature, but there are still receptive fields that are binocularly represented, and additional binocular fields are added. Evidence for binocular development was obtained using evoked potentials and behavioral measures. We are using this information to develop measures that will be used to assess clinical populations.

551.10 DEVELOPMENT OF DISPARITY SENSITIVITY IN A CORRELATIONAL-BASED NETWORK MODEL OF THE VISUAL CORTEX REQUIRES TWO PHASES. GS Berns, P Dayan and TJ Sejnowski*, The Salk Institute, La Jolla, CA, 92037.

A correlational-based model of development of disparity sensitivity in the visual cortex was simulated. Two one-dimensional input layers, representing retinal and thalamocortical inputs from each eye, were full connected to a single one-dimensional cortical layer with fixed intra-cortical connections. The weights were modified by a linear Hebb rule using correlations both within and between eyes and were adaptively normalized. Weights that reached zero were frozen. Three developmental paradigms were investigated: 1) retinal activity locally correlated within each eye but not between eyes, which might occur during prenatal development; 2) retinal activity locally correlated both within and between eyes, which might occur during postnatal development; and 3) two-phase development with the first phase corresponding to paradigm 1 and the second phase corresponding to paradigm 2, modelling both pre and postnatal development. The development of disparity and ocularity are intimately linked in our model. With no between-eye correlation, the cortex developed only monocular cells without any disparity sensitivity. Between-eye correlations throughout development led to a cortex of uniformly binocular cells with the receptive fields of both eyes aligned and thus tuned to zero disparity. The two-phase paradigm allowed the initial development of a monocular bias which was partially reversed by the addition of between-eye correlations. This resulted in a cortex populated by both monocular and binocular cells, the binocular cells tending to have zero disparity and the more monocular cells having nonzero disparity, thus matching the experimentally observed relationship of disparity and ocularity in the cat (LeVay & Voigt, Vis. Neurosci. 1988). (Supported by the Howard Hughes Medical Institute and SERC.)
VISUAL DEVELOPMENT: STRIATE CORTEX II


Release of γ-aminobutyric acid (GABA) was measured by brain microdialysis and high-performance liquid chromatography in the visual cortex of anesthetized kittens. First, we confirmed that visual stimulation gave rise to a marked increase in GABA release which was completely suppressed by infusion of tetrodotoxin (TTX). However, GABA release induced by nipeicolic acid, a GABA uptake inhibitor, was unaffected by the TTX infusion. Next, we examined the effect of local infusion of noradrenaline (NA) on the release of GABA. Exogenous NA caused a complete suppression of visually-induced GABA release as well as basal release of GABA. These results suggest that cortical GABA release may play a role in the NA-induced ocular dominance plasticity that cortical infusion of NA caused an obvious shift of ocular dominance in favor of the eye exposed monocularly to moving visual stimuli in the kitten visual cortex (Imamura and Kasamatsu, Exp. Brain Res., 1991).

552.2 THE POSTNATAL DEVELOPMENT OF EXCITATORY AMINO ACID BINDING SITES IN FERRET VISUAL CORTEX. A.L. Smith and I.D. Thompson (SPON: Brain Research Association). University Laboratory of Physiology, Oxford University, Parks Road, Oxford OX1 3PT, U.K.

We have used a combination of tranenural tracing techniques and quantitative in vitro autoradiography to investigate how the distribution and levels of EAA binding sites in cortex correlate with the establishment of geniculocortical connectivity in ferrets. Intracranial injections of wheatgerm agglutinin horseradish peroxidase showed that geniculate afferents were present in subplate at birth, had started to enter the cortical plate by P16 and exhibited an adult-like laminar distribution in cortex at P30. Radioligand binding studies, performed on cryostat sections, showed marked differences in the expression of kainate, AMPA and NMDA binding sites. Kainate binding sites (labelled with [3H]kainic acid) are highly localized throughout development and are found mainly deep in the cortical plate or in the subplate. AMPA binding sites (labelled with [3H]AMPA), however, are widely distributed in the developing ferret brain and before eye-opening exhibit a much more homogeneous distribution in the cortical plate than kainate binding sites. A variety of radioligands ([3H]guaianic acid, [3H]UCP-39653 and [3H]MK-801) were employed to localize NMDA binding sites and these gave rise to different distributions implying a heterogeneity in the binding sites for these radioligands. NMDA binding sites are only present at very low levels on the day of birth and throughout early development and they exhibit a more homogeneous distribution in cortex than non-NMDA binding sites. Shortly before eye-opening NMDA binding site levels increase dramatically. Taken together these findings suggest that the subtypes of EAA binding site play distinct roles in regulating thalamocortical connectivity during development.

This work was supported by The Welcome Trust, U.K.

552.3 PATCHY EXPRESSION OF C-FOS IN AREA 17 OF KITTENS. C. Beaver, K.M. Murphy and D. E. Mitchel*. Dalhousie University, Halifax N.S. and McGill University, Montreal, CANADA.

Physiological and anatomical investigations of area 17 of cats have revealed a system of functional modules or columns based upon a salient visual response characteristics of neurons, namely ocular dominance and orientation preference. Both systems of columns exist in an immature state at birth, but develop rapidly afterwards in normal kittens to attain adult-like anatomical form by about 6 wks of age. The postnatal development of both systems of columns requires visual experience since their development is both arrested in kittens reared from birth in total darkness and their pattern is altered in animals that receive biased early visual experience. Recent results obtained from the use of other anatomical methods (such as cytochrome oxidase histochemistry) on cat visual cortex point to the possible existence of other principles of modular organization in addition to those uncovered earlier. In order to investigate whether any system of modular cortical organization arises by factors intrinsic to the cortex itself we have exploited the recent observation of rapid expression of the immediate-early gene, c-fos, in the visual cortex of area 17 of normal kittens following very brief exposure to light. We have examined the tangential distribution of c-fos in kittens reared from birth in total darkness until 30d and then allowed 1-2 hrs of either monocular or binocular visual exposure. The positive cells were labeled immunohistochemically and visualized in tangential sections from unfolded and flattened visual cortex. Surprisingly, distinct patches of c-fos expression were evident in layers 2 and 3 of area 17 of binocularly reared kittens as well as monocularly reared kittens. The arrangement of the patches of c-fos expression were compared to the modular structure revealed by other immunohistochemical methods. However, the fact that c-fos expression in these dark-reared kittens is no so-uniform suggests that at least some aspects of cortical modularity may be intrinsically determined.

552.4 AGE DEPENDENCE OF MK-801 BINDING SITES IN VISUAL CORTEX. TISSUE SPECIFICITY, BINDING SITE SPECIFICITY, AND CHANGE IN PROPERTIES. B. Gordon*, Y. Tseng, and K. Towar. The number of MK-801 binding sites in cat visual cortex is maximal at about 42 days (d) of age. This fact, among others, suggests that these receptors may be involved in visual cortex plasticity. We asked whether this age dependence of MK-801 binding sites is specific both to visual cortex and to the MK-801 binding site. To examine tissue specificity we compared MK-801 binding in visual cortex, retina and hippocampus. We selected retina because it is never plastic and hippocampus because it is, presumably, plastic throughout life. Retinal binding was very low and did not vary consistently with age. Hippocampal binding increased significantly from 7d to 42d and decreased slightly, but not significantly, in adulthood. Thus, development of MK-801 binding parallels the critical period in both visual cortex and hippocampus. The statistically significant decreases with increasing age occur only in visual cortex. To examine binding site specificity we compared the development of MK-801 and AMPA binding in visual cortex. AMPA binding increased from 7d to 42d, but did not decrease significantly in adulthood. Therefore, the binding peak at 6 weeks may be specific to the NMMA receptor. To determine whether the properties of the MK-801 binding site varied with age, we examined the age dependence of the ability of glutamate, glycine, and speramine to enhance MK-801 binding. Preliminary data suggest that addition of a second enhancer may be more effective in adults than in younger animals, suggesting that the properties of the binding sites vary with age.

552.5 DEVELOPMENT AND REGULATION OF ALPHA ADRENERGIC RECEPTORS IN KITTEN VISUAL CORTEX. Wei-Guo Jia, Yu Lin Liu and Max Cynader. Department of Ophthalmology, University of British Columbia, Vancouver, B.C. Canada V5Z 3N9

Alpha 1 and alpha 2 adrennergic receptors were localized in developing cat visual cortex by using [3H]prazosin, and [3H]fluoroseline, respectively. Effects of neuronal activity on development of the two receptor subtypes were also studied in animals with lesions at various sites of central visual pathway. Binding densities for both ligands increased during the first postnatal weeks and declined thereafter. For both receptor subtypes, the highest concentration of binding sites was found in the subplate zone of the neocortex, then concentrated in cortical layer IV beginning at postnatal day 30 and finally in the superficial cortical layers in adulthood. The development of [3H]prazosin binding was more sensitive to changes in expression than the cortical layer. However, the developmental redistribution of c-fos receptors began earlier than that of the α2 sites. Quinolinic acid lesions within cortex, lesions of the lateral geniculate nucleus and of optic tract reducted binding of both [3H]prazosin, [3H]fluoroseline to various extents in the cortex. Our results suggest that the two α-adrennergic subtypes were mainly located on cortical cells and their lamination is age-dependent and the density of the receptors is regulated by neuronal activity.
Transient overexpression of IP3 receptors during the critical period for kitten visual cortex plasticity. Y.L. Liu* and M.S. Cyndar. Department of Ophthalmology, University of British Columbia, Vancouver, B.C., Canada V5Z 3N9.

Inositol 1,4,5-trisphosphate (InsP3), a second messenger generated via receptor-stimulated hydrolysis of phosphatidylinositol 4,5-bisphosphate, mediates calcium mobilization from intracellular stores. Previous studies in kitten visual cortex have shown that neurotransmitter/modulator receptors linked to PI turnover are consistently concentrated in cortical layer IV during the postnatal critical period for kitten visual cortex plasticity. In order to investigate the correlation of this second-messenger molecule with the molecular basis of visual cortex plasticity, we used an antibody directed against a purified InsP3 receptor glycoprotein of relative molecular mass 260,000 (Ross et al., Nature, 1985) to visualize InsP3 receptors in kitten visual cortex. InsP3 receptor immunoreactivity was found to be concentrated in the pyramidal cells of the deep cortical layers in the first two weeks after birth, and was present in pyramidal cells of layers III, II and V in the third and fourth weeks. Between postnatal days 40 to 60, InsP3 receptor immunoreactivity was most highly expressed in the cortex. Immunoreactive cells were present in all cortical layers, and were most dense in layers VI, IVb and III. After postnatal day 90, the number and intensity of InsP3 receptor immunoreactive cells decreased gradually until adulthood. In adult cat visual cortex, immunoreactive cells were most concentrated in the superficial and deep cortical layers. These results show that InsP3 receptor immunoreactivity is transiently concentrated in different populations of cortical cells during postnatal development and that the overall expression peaks during the critical period. The relationship between the transient expression of the InsP3 receptor and the developmental alteration in cell surface receptors that evoke PI turnover is under study.

**SEQUENCE ANALYSIS OF cDNA CLONES SELECTIVELY EXPRESSED DURING THE CRITICAL PERIOD FOR VISUAL CORTEX DEVELOPMENT.** Shiv Prasad* and Max S. Cynader Department of Ophthalmology, University of British Columbia, Vancouver, B.C., V5Z 3N9.

During the first few months of a kitten's life, the structure and function of the cortex can be dramatically altered as a result of abnormal visual input. During this critical period, which peaks at about 30 days of age, studies at the protein level have documented transient elevation of several important molecules within cortical cell populations.

We have isolated cDNA clones of mRNAs whose levels are higher during the critical period of development in kittens than in adult cats. Among approximately 12000 cDNA clones from a 30-day-old kitten visual cortex cDNA library, 200 were identified by screening with a probe of the 3' untranslated region of a corticotropin-releasing hormone (CRH) cDNA. About 150 of these cDNA clones from the single-copy cDNA clones with northern blots and found that 49 of these represent mRNAs whose levels are much higher in the 30-day-old kitten visual cortex than in the adult visual cortex. Partial nucleotide sequences for about 100 of these cDNA clones have been determined in order to search the EMBL DNA database for identities of these clones. Twenty-two of these clones were identifiable. Their identities showed that these sequences are involved in cell-cell communication, cellular remodelling, neurotransmitter release and processing, neurofilament assembly, energy metabolism, and RNA and protein synthesis.

**ISOLATION AND CHARACTERIZATION OF cDNA CLONES OF mRNAs SELECTIVELY EXPRESSED IN KITTEN LATERAL GENICULATE NUCLEUS.** L.S. Bhargav, S.S. Prasad, R.M. Douglas* and M.S. Cynader. Department of Ophthalmology, University of British Columbia, Vancouver, B.C., Canada.

During the first few months of a kitten's postnatal development, monocular visual deprivation causes marked effects on the morphology, growth, and connectivity of cells in the lateral geniculate nucleus (LGN) connected to the deprived eye. The same manipulation performed in adult animals has no effect. To understand the molecular mechanisms underlying the age and use-dependent plasticity in this nucleus we have used the method of subtractive hybridization to isolate genes that are differentially expressed in the LGN of 30-day-old kittens and not in adult cats.

Subtractive hybridization is a powerful technique for isolating differentially expressed mRNAs. A potential limiting factor for proper subtraction, however, is the requirement of large amounts of driver mRNA. We have utilized a subtractive hybridization protocol with modifications designed to avoid this problem. This method relies on a) Directional cDNA cloning b) Large scale in vivo phagemid excision and c) Generation of bacteriophage run-off transcripts.

Thus far we have isolated five cDNA clones from a 30-day-old kitten LGN cDNA library that appear unique to kitten LGN. Characterization of the clones by Northern and DNA sequence analysis is underway.

**DEVELOPMENTAL EXPRESSION OF bFGF IN THE CAT VISUAL CORTEX.** J. Colyer* and M.S. Cyndar. Department of Ophthalmology, Univ. of British Columbia, Vancouver, B.C., Canada V5Z 3N9.

bFGF is an important neurotrophic factor that modulates glial cell differentiation and function, neuronal maturation, survival and growth of neuritic processes. It affects glial cell motility and migration, regulates protein synthesis and is also a major trophic factor operating at all stages of embryogenesis. It is known that bFGF is widely distributed in CNS and PNS as well as other tissues.

In this study, we immunohistochemically localized bFGF in the cat visual cortex. We used rat anti-bFGF (J/1000 dilution) and examined its distribution in microscopic sections of different age kitten cortices. We showed that bFGF was heavily concentrated in glial cells in the white matter, the subcortical plate and the deep cortical layers of the gray matter in young kittens. Overall immunoreactivity peaked in 10 days old kittens. In 0 and 10 day old kittens bFGF staining was also shown in growth cones and neural processes in the visual cortex. In 20 and 30 day old kittens bFGF was abundant in the white matter and layer 1 of the gray matter. Its concentration showed a dramatic decrease in older age animals in which it was homogenously distributed throughout the cortex.

We studied the cellular distribution of bFGF with double-labeling immunohistochemistry, using GFAP as an astrocyte marker. Although it showed co-localization with GFAP (proving that it is localized to astrocytes), its distribution was not restricted solely to astrocytes. The binding of bFGF with microglia in this system is still under investigation.

These results show that bFGF is overexpressed in young kittens prior to the critical period, suggesting that it may modulate glial proliferation, cell migration and may help to set up the cortex for activity dependent plasticity.


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**THURSDAY PM**

**VISUAL DEVELOPMENT: OPTIC TECTUM**

**1309**

**VISUAL DEVELOPMENT: OPTIC TECTUM**

**THURSDAY PM**


**553.3**

NMDSA RECEPTOR CONTRIBUTION TO EPSCS IN THE DEVELOPING FROG OPTIC TECTUM. S. Witte* and H. T. Cline. Dept. of Physiology and Neurobiology, University of Connecticut Health Center, Farmington, CT 06032.

The NMDA receptor has been implicated in a variety of neuronal plasticity phenomena, including long term potentiation, spatial memory, and activity dependent sorting of sensory afferents. Refinement of retinotectal projections in the frog is blocked by chronic application of the NMDA receptor antagonist APV. However, the sorting of nasal from temporal retinotectal fibers, occurring earlier in development, has been shown to be activity independent. We hypothesize that the NMDA receptor activation may contribute differentially to EPSC's recorded from optic tectum neurons of tadpoles at different developmental stages.

Whole cell patch clamp recordings were made from a region in the middle third of the optic tecta of isolated superfused brains from Rana pipiens tadpoles. The perfusate contained 1.5 mM Ca2+ and 2.5 mM Mg2+ to reduce polysynaptic activity. Recorded neurons were labelled with biocytin or biocytin for later examination of their morphologies to seek possible correlations with morphological findings. The stimulation of the optic nerve was delivered through a suction electrode attached to the nerve stump. Several neurons appeared to have only indirect retinotectal connections. The majority of neurons displayed monosynaptic responses to optic nerve stimulation. For these neurons, separate current voltage curves were constructed from the early and late components of recorded EPSC's. Preliminary data show that in younger animals (stages III and IV) the late, voltage dependent component was very small compared to that in older animals (stage VIII). It appears that NMDA receptors contribute increasingly to retinotectal responses as tectal development proceeds.

(Supported by McKNIGHT Foundation for Neuroscience.)

**553.4**


The polysialylated form of the neural cell adhesion molecule NCAM (PSA-NCAM) has been shown to reduce membrane-membrane adhesion and thus may be permissive for regeneration of axonal connections. We have therefore compared the amount of PSA-NCAM in the Xenopus optic tectum, as revealed by immunostaining, with the activity-dependent mechanisms associated with plasticity. Using an monoclonal antibody to PSA-NCAM, we have found that the amount of staining in the superficial layers of the tectum is low in normal tadpoles, dark-reared tadpoles, and NMDA-treated tadpoles, all of which show high levels of plasticity. These results suggest that the activity-dependent mechanisms associated with plasticity may affect connectivity in part by altering expression of variants of NCAM.

(Supported by US P. H. S. Grant EY-03470 to S. B. U.)

**553.5**

CHARACTERIZATION OF PROTEIN KINASES IN THE DEVELOPING OPTIC TECTUM. A.S. Chocca, L. O. Doss, and M. Comenius-Paton. Yale University, Department of Biology, New Haven, Connecticut.

In order to maintain retinotectal topography the synapses between retinal ganglion cells and their targets in the Rana pipiens tectum are being broken and reformed continuously throughout tadpole life. The notion of this topography is dependent on N-Methyl-D-aspartate (NMDA) receptor activation. Such synaptic flux may contribute to the development of the retinotectal connections model system for examining molecular mechanisms associated with plasticity of synapse formation. Using an in vitro kinase assay we have examined properties of the cyclic AMP-dependent (cAMP), calcium and calmodulin-dependent (CaM-II) and calcium and phospholipid-dependent kinases (PKC) in this tissue. Tectal kinase activity is sensitive to the mammalian enzymes, indicating that the amphibian kinases are functionally related to their mammalian counterparts. Additionally, we have investigated the effects of glutamate receptor activation on protein phosphorylation in intact tectal tissue. Fifty μM glutamate stimulates the phosphorylation of 12 proteins that we could identify as specific kinase substrates from our in vitro assays. Five have molecular weights and isoelectric points similar to proteins identified as CaM-II substrates, 3 of which show high levels of plasticity. These results suggest that the activity-dependent mechanisms associated with plasticity may affect connectivity in part by altering expression of variants of NCAM.

(Supported by U.S. Dept. of Education and Training Grant #14606.)

**553.6**

DEVELOPMENT OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE OPTIC TECTUM OF TADPOLES. E.A. Debski* School of Biological Sciences, Univ. of Kentucky, Lexington, KY 40506.

In adult Rana pipiens, somatostatin-like immunoreactive (SOM-IR) tectal cells are found mainly in the caudal third of the optic tecta (Debski, Neurosci. Abstr. 17:1134, 1991). This non-uniform distribution implies that different regions of the tectum may be composed of functionally non-equivalent cells. To begin to understand what function(s) these SOM-IR cells may have, I investigated their distribution in tadpoles.

In stage XIX tadpoles, where caudal regions of the tectum are largely developed, the number and location of SOM-IR cells are about the same as in the adult. Neurites from some of the stained cells in layers 4 and 6 can be followed radially into layer 7 where they turn and extend horizontally. This morphology indicates that at least some of the SOM-IR cells may have efferent projections. In stage XIII tadpoles much of the caudal tectum is immature. Nevertheless, SOM-IR cells present in much greater numbers than in the adult, are largely restricted to these regions. In caudal tectum that has a thickened but as yet un laminated cellular layer, the cells are found in radially oriented columns throughout the layer. Within the thin caudal regions of tecta that are the least mature, SOM-IR cells are absent. In the caudal tectum of the adult, SOM-IR cells are found in the caudal recess and in the ventricular zone. Staining of neurites has not been observed at this stage. These data suggest that SOM-IR tectal cells are a late developing population of cells that may have function.

(Supported by BRSG SO7 RR07114-22 and FFS GA91079.)
554.1
DENDRITIC DEVELOPMENT OF LGN CELLS DURING LAMINAR AND SUBLAMINAR RETINOTEMPORAL SEGREGATION IN THE FERRET. M. Rochat* and M. Sur. Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139; and Biophysical Institute, Federal University of Rio de Janeiro, 21941, Brazil.

The ferret LGN undergoes profound morphological changes during the first postnatal month. Retinotopically aligned single neurons first appear in eye-specific laminae and then into on/off sublaminae during the first and third postnatal weeks, respectively. Here, we have examined how LGN cells acquire their mature dendritic form during this period. Ferrets between postnatal day 1 (P1) and 28 were used. Rhodamine-labeled latex microspheres were injected into primary visual cortex to label LGN relay cells. The detailed morphology of these cells was then revealed by intracellular injections of Lucifer Yellow in 300-μm horizontal thalamic slices kept alive in a tissue-slice chamber. At P1, LGN cells were morphologically immature, with small dendritic arbors and few branches (mean: 5±6. dendritic area: 2.22±1.0 μm²; 70±4 branch points/cell). By P7, dendritic area increased several times (17±6.6 μm²; 10±4 branch points/cell), distributed in a stellar or oriented pattern, and were covered with dendritic appendages (30 ±9 branch points/cell). A more elaborate pattern of dendritic branching was observed in dendritic area: 25.61±11.3 μm²; 50±13 branch points/cell), and spine density had increased significantly (45 ±10 spines/cell). By P21, cells appeared adult-like in structure (dendritic area: 33.6±18.9 μm²; 40±12 branch points/cell). Cells at P28 were similar in morphology, with dendritic area: 29.85±10.3 μm²; 38±9 branch points/cell). We conclude that developing LGN cells undergo considerable dendritic remodelling to reach their mature morphology, attaining most of their adult features by P21. This indicates that dendritic development is correlated with the formation of appropriate neurotransinaptic connections. Supported by EY07023 and CNPq.

554.2
RAPID INCREASE IN ASTROCYTIC GFAP IMMUNOREACTIVITY IN LGN OF ENucleATED RATS. K.S. Candy*, J.F. Olavarria & E.W. Rubel. Virginia Merrill Bloedel Hearing Research Center and Dept. of Psychology, Univ. of Wash., Seattle, WA 98195

We have previously found that the cessation of action potentials in the cochlear nerve (by deafenation or TTX treatment) results in increased immunoreactivity for glial fibrillary acidic protein (GFAP) in the brainstem reticular nuclei within 1-3 hours (J. Neurosci. 12:1001,1992; J. Comp. Neurol. 316:415,1992). To determine whether this rapid astrocytic response to neuronal inactivity could be examined in the rat LGN at short times after unilateral enucleation.

Anesthetized rats were perfused 3, 6 or 12 hours following eye removal. Paraffin sections containing the LGN were processed immunohistochemically using a polyclonal antiserum to GFAP. Area density of GFAP-IR in the rat LGN at short times after unilateral enucleation.

We report here that phasic PGO waves are associated with a rapid increase (≥150%) in the density of GFAP-IR. By P21, cells appeared adult-like in structure (dendritic area: 33.6±18.9 μm²; 40±12 branch points/cell). Cells at P28 were similar in morphology, with dendritic area: 29.85±10.3 μm²; 38±9 branch points/cell). We conclude that developing LGN cells undergo considerable dendritic remodelling to reach their mature morphology, attaining most of their adult features by P21. This indicates that dendritic development is correlated with the formation of appropriate neurotransinaptic connections. Supported by EY07023 and CNPq.
VISUAL DEVELOPMENT: MAMMALIAN THALAMUS AND MIDBRAIN

1311

Routine immunocytochemical techniques were used to localize the calcium-binding protein, calbindin-D 28K (CaBP) within the developing cat pretectum. Brains fixed in Bouin’s were embedded in paraffin and cut in one of three stereotaxic planes. Tissues were sampled from neonates and postnatally at 7, 14, 28, and 56 days. CaBP positive cells appeared to be randomly distributed within the nucleus of the optic tract (NTO), posterior (NPP), and medial pretectal nucleus (MPP). Within the anterior pretectal nucleus (NPA), CaBP positive cells could not be easily separated into the two subdivisions, NPAc and NPAr, however, there was a distinct ring of CaBP stained tissue along the border of NPA. The percentage of cells staining for CaBP either remained level or generally increased postnatally for NTO, NPA, NPP, and NPM, with the maximum cellular immunoreactivity never exceeding 26%. Of particular interest, however, was the pretectal olivary nucleus. Neurons staining positive for CaBP numbered approximately 50% for the first two weeks of neonatal life, dropping to approximately 25% after two months of development. These preliminary data would suggest that the olivary nucleus is under considerably different developmental influences than the other four pretectal nuclei. (Supported by NEI EY00777)

DEVELOPMENTAL REGULATION OF NITRIC OXIDE SYNTHASE mRNA AND NEURONAL NADPH DIAPHORASE ACTIVITY IN THE RAT SUPERIOR COLICULUS. G. Pruszky*, M. Hoffer and M. Constantin-Paton. Department of Biology, Yale University, New Haven, CT 06511.

In rats, there is a topographical refinement of the retinal projection to the superior colliculus (SC) during development which is dependent upon NMDA receptor activation (Prusky et al., 1991). Since NMDA receptor activation can lead to the production of nitric oxide (NO), a messenger molecule that has been proposed as a diffusible retrograde signal at Hebbian synapses (Galley et al., 1990), we are investigating a potential role for NO in retinocollicular plasticity. Using a CDNA probe that recognizes NO synthase (NOS) (Bredt et al., 1991), we have employed quantitative Northern blot analysis of the rat superior colliculus during postnatal development. NOS mRNA levels were very low during the first postnatal week, and increased moderately by postnatal day (P)12. The highest NOS mRNA levels were observed at P19. By adulthood, NOS message was reduced by 50% from levels detected at P19. Since NOS appears to be identical with neuronal nitric oxide synthase (NOS), we have also used histochemistry of this enzyme as a marker of age-related alterations in NOS activity. There was no detectable NADPH diaphorase activity during the first postnatal week, but faint staining of neurons in the superficial SC could be detected at P12. By P19, NADPH diaphorase positive neurons were more abundant and were more intensely labelled. This trend continued and reached its peak by P27 when many neurons of different cell classes were labelled in the superficial SC. In the adults, the overall level of NADPH diaphorase reaction product in the superficial SC was lower than at P27. NOS markers have similar developmental profiles to NADPH message (Hoffer et al., this meeting), and may indicate a relationship between NO production and NMDA receptor expression in the control of collicular synaptic plasticity. Supported by NEI EY00909 to MCP, and MBRC and EMBO fellowships to GM and MH, respectively.


The development of the retinocollicular projection in the Brazilian opossum, Monodelphis domestica was examined from birth (P0) until P30. Monodelphis is a small, pouchless marsupial which breeds well under laboratory conditions and whose young are born in an extremely immature state. Retinal ganglion cell (RGC) axons were anterogradely labeled by placing crystals of the axon tracer DiI into the eyes of aldehyde-fixed specimens.

The eyes of P0 opossums were relatively immature and immunocytochemical localization of immunoreactive axons of differentiated RGCs in the only present in 1/3 of the developing eye. In P1 opossums the contralateral projection has grown across the chiasm and proceeded a short distance into the optic tract. By P2, RGC axons have grown approximately 2/5 the distance up the side of the midbrain towards the superior colliculus (SC). In P3, a smaller number of optic axons were observed. RGC axons have reached the SC by PNS5/7 and have begun elaborating axonal arborizations by PNS8.

Examination of P1 rats revealed numerous varicosities as well as side branches emanating from many of the RGC axons. Growth cones were identified in a number of specimens at various ages, displaying a variety of morphologies. Axons pioneering the retinal projection usually terminated in morphologically complex growth cones. We have also observed DiI labelling of growth cones, suggesting the presence of inter-retinal projections at these early stages of development.

Since a great deal of the development of the visual projection occurs postnatally in Monodelphis, this species is likely to provide an excellent system for in vivo experimental manipulations and analysis.


The uncrossed retinocollicular pathway in normal rodents terminates deep within the upper grey layers and there is no binocular segregation in the tectum. We studied the retinotectal projections in rats using anterogradely transported horseradish peroxidase, after changing the balance between the two pathways originating from both eyes as a consequence of either contralateral optic tract lesions (OTL) or small temporal retinal lesions (TRL) made at birth (P0). After left OTL an aberrant uncrossed projection develops at the surface of the right tectum from both eyes at the time of birth. At P10 this projection is fully developed and binocular segregation starts to appear. At P14-16% of the animals had developed a crossed pathway from both eyes at the surface of the right tectum, which was most prominent at the surface. Similar, though progressively smaller, rearrangements were obtained after TRL at P5-21. We conclude that both laminar selection and segregation of the retinocollicular pathways depend on a critical balance between the inputs from both eyes, which can be modified by a prolonged period of development.
dorsal/ventral polarity, but afferents from these retinae show no such pattern. This indicates that the increased tectal area for each retina allowed them to make contacts in the tectum than they do in the tecta of normal hamsters. However, the bouton density (N=1312, m=15; E=1616, n=15; t=2.12, df=28, p<0.05) and branches (N=2.27, m=15; E=2.83, n=6; t=2.125, p<0.05) of the retinal axon arbors in the encased animals were lower, suggesting there is an intrinsic limit to the number of boutons and branches that these axons can form.

Our previous study showed that retinal ganglion cells can reduce their arbor size by almost half to compensate for a decrease in available tectal area and remain viable. This study shows retinal gangion cells can support greatly increased arbors, but with markedly altered clustering of terminal areas along their increased length. Supported by NIH NS 19245.

554.13 INNERVATION OF THE SUPERIOR COLLIQUUS BY NASAL OR TEMPORAL RETINAL GANGLION CELL AXONS FROM ECTOPTIC HEMIRETINA. K.T. Yee* and R.D. Lund. Department of Neuroanatomy, Anatomy and Cell Sciences, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261 and Department of Anatomy, University of Cambridge, Cambridge, CB2 8EF, U.K.

Ectopic retinial introduced into postnatal hosts maintain intrinsic dorsoventral polarity, but afferents from the retinae show a topographic ordering in their projection to the primary target, the host superior colliculus (SC) (Yee and Lund, ’91), although synapses are not able to mediate behavior through this host animal (Lund and Yee, ’91). We have postulated that markers for establishing topography may no longer be present when ectopic retinial afferents arrive in the SC. Welsh et al. (’97) show that temporal, but not nasal retinal axons are repelled, in vitro, by posterior tectal membranes; this mechanism may be important for map formation in the tectum. We have examined whether a similar mechanism is operative, in vivo, following retinal transplantation.

Retiniae transplanted to the midbrain were used to examine whether innervation patterns from nasal and temporal retinal ganglion cell axons differ. Nasal or temporal hemiretiniae from embryonic day 12 mouse donors were transplanted into each of eight SC of postnatal-day 1 rat hosts. Animals were sacrificed at 3-5 weeks of age and midbrain sections were stained with a mouse specific antibody (anti-M4) to identify projections from the transplanted mouse retina.

Afferents from both nasal and temporal hemiretiniae innervate the caudal aspect of the SC more heavily than the rostral portion, and show different patterns of innervation which reflect the region of retina transplanted. While the hemiretiniae show regional specificity of innervation, innervating only visual target nuclei of the host, mechanisms governing the establishment of normal retinotopy do not appear to be operative.

Supported by NIH EY 05308 and Action Research.


Maturing corticocortical axons undergo morphological changes that parallel development of synapses in the superior colliculus. To correlate morphological changes with synaptic development, electron micrographs were used to serially reconstruct biocytin-labeled axons and their contacts. Biocytin was injected into the visual cortex of cats aged 0 to 35 days. The tissue was processed to visualize the biocytin.

At the earliest age the labeled corticocortical axons were thin and unbranched with periodic dilations. These dilations contacted multiple vesicle-filled profiles that were absent along the lengths of axons lacking dilations. Profiles contained either clear pleomorphic vesicles or both clear pleomorphic and dense-core vesicles. Maturing labeled axons developed short side branches with presynaptic vesicles and contacted vesicle-filled postsynaptic profiles with synaptic clefts. Later axons showed elaborated arborizations with greater numbers of presynaptic vesicles and synaptic clefts that contacted dendritic profiles with fewer postsynaptic vesicles. Thus, vesicle-filled dendrites appear to interact with axonal dilations prior to the initiation of synapse formation and may guide local axonal reorganizations, and synapse selection and formation.

555.1 MOLECULAR DIFFERENTIATION OF GANGLION CELLS IN NORMAL AND OCULAR RETRACTION MOUSE RETINA. M.J. Hack*, F. Herring, and D. Goldberg*.

We are interested in molecular mechanisms underlying retinal ganglion cell (RGC) development in normal and ocular retraction (OR) mice. Since RGCs (which are driven to type or the visual target nuclei of the host) are innervating only visual target nuclei of the host, mechanisms governing the establishment of normal retinotopy do not appear to be operative.

Supported by NIH EY 05308 and Action Research.

555.2 AXOTOMY-INDUCED EXPRESSION OF IMMEDIATE EARLY GENES IN RAT RETINAL GANGLION CELLS. G.A. Robinson* and A.R. Light. Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

To assess the effect of axotomy on retinal ganglion cell (RGC) expression of immediate early genes (IEGs), we retrogradely labelled RGCs with Fluoro-Gold (FG) applied to their targets in vivo. We have assayed the retina for genes which are induced during RGC differentiation: gene expression was detected by in situ hybridization with cRNA probes for GAP-43, nAChR alpha-3 and beta-3 subunit genes (markers of RGCs which have migrated to their final positions); and expression was decreased in mesencephalohyochromatolytic antibodies against neuron-specific beta-tubulin (Tuft1) and a neuronal cell adhesion molecule mediating interactions between RGC axons (L1), both of which are expressed in early retinogenic retinogenic retinae. In mouse retina, embryonic day 11 (E11) is the first day at which RGCs become postmitotic. At E11 we detected expression of beta-tubulin (in soma and axons) and L1 (on axons) in cells spanning from the central retinal sheet to the central vitreal surface. In contrast, beta-tubulin was not detected in the OR retina until E12, implying that RGC development may lag behind that in normal mouse retina. Nicotinic AChR and GAP-43 gene expression was induced in cultured RGCs at E12 in normal retina; by E13 the borders of nAChR and GAP-43 gene expression had spread peripheral to the retinal midline. In striking contrast, nAChR alpha-3 and beta-3 subunit genes were not induced in OR RGCs, while the GAP-43 gene was expressed in a manner consistent with the normal retina.

These results indicate that RGCs develop in the OR mouse, as revealed by positive Tuft1, L1 and GAP-43 gene expression in OR ganglion cell nuclei in the normal retina. In the OR retina, nAChR gene expression in ganglion cells is consistent with the hypothesis that an interaction between a RGC axon and a brain target is responsible for nAChR gene induction. In addition, these results suggest that the mechanisms regulating the expression of temporally correlated ganglion cell-specific genes proceed along diverging pathways.
555.5

REGENERATING RETINALGANGLIONCELLAXONES BRIDGED BY PERIPHERAL NERVE GRANTS CAN EXTEND INTO THE BRAIN STEM THROUGH THE TRIGEMINAL NERVE ENTRY ZONE. R. Pallini1, E. Fernandez2, L. Lauretti1, V. Borzini1 and A. Murri1,2,3,4,5.

Institutes of Neurosurgery and Neurology (1), Catholic University, Rome, Italy.

Axotomized retinal ganglion cells (RGC) of adult mammals can regenerate their cut axons over long distances into peripheral nerve (PN) grafts (Vidal-Sanz et al, J Neurosci 7:2894-2909, 1987). The regenerating RGC axons can be directed into the PN graft to selected central targets. However, most cells in PN grafts survived are blocked by the gial scar at the interface between the PN and the CMS. In the present study, we develop a new model, 1) specifically at increasing the penetration of the regenerating central environment. The optic nerve (ON) of adult rats was cut intracranially and an acellular nerve substitute (40 mm) was sutured to the ON stump. After a complete histological retrograde analysis, the other end of the PN graft was resected to avoid the central stumps of the regenerating nerve (TM). Three months after grafting, the regenerating RGC axons were labeled by retrogradely transported horseradish peroxidase (HRP) (WGA-HRP, Sigma, &8 solution) injected into the graft (0.05-0.2 μl) or vitreous body (1 μl).

We found that the substantial number of the RGC axons that had regenerated into the PN graft, were growing through the TB entry zone and extended into the brain stem for distances of up to 1200-1300 μm. Although many of these axons followed various different positional paths, a preferential growth into the spinal trigeminal tract was seen.

555.6

AN IMMUNOCYTOCHEMICAL MARKER FOR HAMSTER RETINAL GANGLION CELLS. P. G. Bruck1*, W. C. West1*, K. R. Byr1 and D. O. Ernst1.*

1 Dept of Neurology, Massachusetts General Hospital, Charlestown, MA & 2 Center for Biotechnology, Baylor College of Medicine, Houston, TX.

About 50% of the neurons in the ganglion cell layer of the rodent retinas are amacrine cells. Therefore, the RGC cell layer per se does not identify that cell as a retinal ganglion cell (RGC). At present, RGCs can be unequivocally distinguished only by retrograde labeling with neuronal markers. We have demonstrated that the ganglion cell marker, c-Fos, is a reliable marker of RGCs in the retina of adult hamsters.

To overcome this difficulty, we determined if c-Fos, a monoclonal antibody that selectively labels RGCs in other species, would be useful in the identification of RGCs in the hamster retina. We found that c-Fos selectively labels RGCs in developing and adult hamsters if it is injected into the vitreous body. The high level of expression is maintained for over 3 days in developing hamsters and for at least 2 weeks in adult hamsters. Together, these results suggest that c-Fos may serve as a reliable marker of RGCs in the hamster retina.
VISUAL DEVELOPMENT: RETINAL GANGLION CELLS

S. L. Schmidt* (1), J. L. W. F. Viral (2) and B. L. Ideberg (1), Department of Neuroanatomy, Instituto de Biologia da UFRJ (1); Departamento de Ciencias Fisicas, Instituto de Biologia da UERJ (2).

Prenatal ionizing irradiation of pregnant mice produces in the progeny extensive shrinkage of the posterior halves of the cerebral hemispheres and the dorsal lateral geniculate nucleus (dLGN). In this study we describe the retinal projections to subcortical nuclei in adult mice irradiated prenatally. Pregnant mice were exposed to a gamma source at 16 days of gestation receiving a total dose of 30Gy. Adult mice (n=13, irradiated progeny; n=13, nonirradiated controls) received an eye injection (5 μl) of a 30% solution of horseradish peroxidase (Sigma type VII). The animals were allowed to survive for 2 days. Then the brains were reacted for peroxidase using tetramethyl-benzidine as a chromogenic substrate. Analysis of the retinal projections indicated that the general pattern of connection of normal and irradiated animals was similar. Bilateral retinal projections were found in dLGN, ventral lateral geniculate nucleus, posterior pretectal nucleus, olivary pretectal nucleus and superior colliculus. Label was found in nucleus of the optic tract only on the side contralateral to the injected eye. In spite of the damage to the striate cortex and the reduction of the dLGN, no abnormal retinal projection fields were detected in irradiated animals. These data differ from those obtained by other authors after neonatal surgical removal of the rat striate cortex. In these cases, aberrant pathways were consistently found. Our data suggest that prenatal or postnatal cortical damage may lead to distinct consequences regarding the redistribution of the retinal axons.

Supported by CNPq, FINEP, FAPESP

VISUAL DEVELOPMENT: ABNORMAL DEVELOPMENT OF CORTEX

556.1

RESTRICTED CALLOSAL CELL DISTRIBUTION IN THE STRIATE CORTEX OF THE RABBIT FOLLOWING SYSTEMIC YOHIMBINE ADMINISTRATION DURING DEVELOPMENT. Yuechu Wang,1 A. M. Grigorieff2 and J. L. W. F. Viral.1,2 *Departments of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129, and 2Department of Anatomy, Hahnemann University, Philadelphia, PA 19120-1192.

In the mature rabbit, the distribution of visual callosal cells which project to the contralateral visual cortex is limited to the 17/18 border. In the newborn rabbit callosal cells are distributed throughout most of the mediolateral extent of area 17. This developmental process of fine tuning in the visual cortex has been shown to be regulated in part by the level of noradrenaline (NA). In the present study we examined the effects of administration of an alpha 2 receptor antagonist, yohimbine, during the critical period, on the development of the visual callosal cell distribution. Yohimbine increases the release of NA by blocking presynaptic alpha-2 adrenergic receptors. Rabbits received i.p. injections of yohimbine HCl (2.5 mg/kg) every day from postnatal day 5 through day 12 (N=4). Rabbits were raised until adult, at which time multiple injections of HRP (Bohringer) at 0.5% in H2O were made into one eye and 0.5% into the other eye throughout one entire visual cortex. Animals were perfused 24 hours later and the brains were cut and reacted with TMB. Adult animals which received yohimbine injections had a significantly reduced tangential extent of the callosal cell distribution in area 17, and also had a significantly reduced callosal cell density in lamina III compared to normal rabbits. Yohimbine administration during the critical period enhanced the process of retraction of exuberant callosal projections. The results provide evidence that NA plays a role in the normal elimination of early exuberant pathways during development.

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556.3


Rabbits received intraperitoneal injections of yohimbine HCI (2.5 mg/kg) throughout one entire visual cortex and 'wait' before entering, while their principal targets, the cells of layer 4, migrate to their final position. At birth, the terminal fields of fibers from the thalamus and adjacent sections were counterstained with neutral red. Analysis of the retinal projections revealed an abnormal pattern of geniculate cell distribution. 

556.4

INTERFERENCE WITH GENERATION OF LAYER 4 PREVENTS THE FORMATION OF OCULAR DOMINANCE COLUMN. Peter Fried, Frank Georgie1, Ulrich Egelhofer and Colin Blakemore. University Laboratory of Physiology, Oxford OX1 3PT, U.K.

In the cat, the LGN fibers arrive under the visual cortex and 'wait' before entering, while their principal targets, the cells of layer 4, migrate to their final position. At birth, the terminal fields of fibers from the two eyes overlap, but they gradually segregate into ocular dominance (OD) columns over the first few postnatal weeks. To investigate the relationship between the generation of layer 4 and subsequent synapticogenesis and formation of OD columns, we used methylazoxymethanol acetate (MAM), a cytotoxin that destroys dividing cells, partially to disrupt the generation of layer 4. Time-mated pregnant cats were given a single injection of MAM (10mg/kg i.p.) on embryonic day 40.5 (the peak of layer 4 cell generation). The pattern of termination and extent of geniculo-cortical segregation were assessed in the adult offspring using transneuronal tracing with WGA-HRP.

Control thickly laminarized animals appeared virtually normal and lamination roughly equivalent to layers 1 and 2/3 was discernible; however, the density of cells in layer 4 was modestly reduced. Moreover, transneuronal tracing revealed an abnormal pattern of geniculate termination in some parts of areas 17 and 18 with dense termination extending up to the lower limit of layer 1. Even years after birth there was no hint of segregation into OD columns. There was also a marked down-regulation of the Cat-301 antigen in the upper and lower layers of areas 17 and 18. These data suggest that the cells of layer 4 play an important role in determining the laminar pattern of axon termination and in regulating the competition that underlies OD column formation. - Supported by the MRC.

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555.9

EFFECTS OF PRENATAL IONIZING IRradiATION ON THE DEVELOPMENT OF MOUSE VISUAL PATHWAYS. S. L. Schmidt* (1), J. L. W. F. Viral (2) and B. L. Ideberg (1), Department of Neuroanatomy, Instituto de Biologia da UFRJ (1); Departamento de Ciencias Fisicas, Instituto de Biologia da UERJ (2).

Prenatal ionizing irradiation of pregnant mice produces in the progeny extensive shrinkage of the posterior halves of the cerebral hemispheres and the dorsal lateral geniculate nucleus (dLGN). In this study we describe the retinal projections to subcortical nuclei in adult mice irradiated prenatally. Pregnant mice were exposed to a gamma source at 16 days of gestation receiving a total dose of 30Gy. Adult mice (n=13, irradiated progeny; n=13, nonirradiated controls) received an eye injection (5 μl) of a 30% solution of horseradish peroxidase (Sigma type VII). The animals were allowed to survive for 2 days. Then the brains were reacted for peroxidase using tetramethyl-benzidine as a chromogenic substrate. Analysis of the retinal projections indicated that the general pattern of connection of normal and irradiated animals was similar. Bilateral retinal projections were found in dLGN, ventral lateral geniculate nucleus, posterior pretectal nucleus, olivary pretectal nucleus and superior colliculus. Label was found in nucleus of the optic tract only on the side contralateral to the injected eye. In spite of the damage to the striate cortex and the reduction of the dLGN, no abnormal retinal projection fields were detected in irradiated animals. These data differ from those obtained by other authors after neonatal surgical removal of the rat striate cortex. In these cases, aberrant pathways were consistently found. Our data suggest that prenatal or postnatal cortical damage may lead to distinct consequences regarding the redistribution of the retinal axons.

Supported by CNPq, FINEP, FAPESP
555.6
CHOLINE ACETYLTRANSFERASE FIBERS IN THE STRIATE CORTEX OF VERTICAL AND HORIZONTAL STRIPE-REARED KITTENS PREFERENTIALLY DEVELOP ORTHOGONAL TO THE SELECTED VISUAL ORIENTATION. Nancy J. Wootl, Laboratory of Chemical Neuroanatomy and Dept. Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

Limited visual experience in young, light-deprived kittens to vertical or horizontal stripes has been shown previously to produce morphological changes in the basilar dendrites of pyramidal cells in striate cortex (Tieman and Hirsch, Comp Neurol. 211: 335, 1982). The present study addressed the possibility of developmental plasticity in one of the presynaptic inputs to cortical pyramidal cells, namely the cholinergic afferent axons. ChAT was measured over a 2-week period, until 5 weeks of age, at which time they were 1) exposed to a vertical stripe pattern, 2) exposed to a horizontal stripe pattern, or 3) given no light exposure. ChAT fibers running parallel were decreased 25 - 68% in layer 5, roughly equal, but in stripe-reared conditions, ChAT fibers running parallel were decreased 25 - 68% in layer 5.
Longitudinal recordings from the primary visual cortex revealed that the number of synapses per unit area determined for deep laminae. As shown below, early and late shunts restored synaptic density to within 32% (p = 0.08) and 35% (p = 0.005) of controls, respectively. Synapses/17.6 9.1 12.0 21.0 14.2 13.8

The percentage of binocular cortical neurones was nearly halved. By contrast, these parameters were within the normal range in Myeloma transplanted rats. We conclude that inhibition of NGF activity affected both the LGN and the visual cortex, suggesting a physiological role for NGF in the development of these structures.

Inhibiting NGF activity caused a delay of recruitment of binocular responses in the visual cortex. However, this activity-dependent modification occurs only within a "critical period" in development and not thereafter. In adult visual cortex, neurons are no longer susceptible to monocular deprivation. To test the hypothesis that NGF may enhance neural plasticity in adult visual cortex, varied doses of NGF were continuously infused, by means of osmotic minipumps, into visual cortex of adult cats who had one eyelid sutured closed at the time of minipump implantation. After various times (minimum 2 weeks), the ocular dominance distributions of neurons in the visual cortex were assessed with single unit recording. We found that there was a dramatic change in ratio of binocularly activated neurons to monocular neurons in the adult visual cortex after monocular deprivation coincident with NGF-treatment. Our results demonstrate that intraocular NGF can recreate the ocular dominance plasticity in adult cat visual cortex. The mechanisms by which NGF induces neural plasticity in adult visual cortex are now under investigation.

NGF nerve growth factor induces neuronal plasticity in adult visual cortex

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557.1 LOCALIZATION AND CHARACTERIZATION OF SPECIALIZED MEMBRANE CONTACTS BETWEEN IMMATURE CONES IN THE PHOTORECEPTOR MOSAIC OF THE FETAL MONKEY RETINA. Kenneth C. Weller and Paolo Rakic. Soc. of Neuroembryology, Yale Univ. School of Medicine, New Haven CT 06510.

Developmental examination of the maturing photoreceptor mosaic in the fetal monkey retina has revealed a rare array of prococious cones that are transiently in apposition with one another (Weller and Rakic, '91, Nature, 351: 397). Previous studies of dissociated cell cultures suggest that the development of photoreceptor phagosomes may be specified in part by early cell-cell interactions that may be mediated by either diffusible factors or through specialized membrane contacts (Watanabe and Rakic, '90, Neuron, 2: 461). To examine the existence of potential contacts between neighboring cones in the primate retina we initiated an ultrastructural and immunocytochemical study of the photoreceptor mosaic at the onset of differentiation of the photoreceptors and bipolar sensitive cone subtypes.

The peripheral region of retina from threes monkeys sacrificed at embryonic (E) days ranging between E50 and E110 were prepared for electron microscopy or immunocytochemistry. Examination of the retina at E50 or E70, prior to the development of outer segments, revealed the presence of punctate specialized membrane contacts between the inner segments of immature cones. At early fetal ages these contacts are unlikely to be gap junctions since neither our electron microscopic or immunocytochemical examination using antibodies specific to the connexin-32 fragment of the gap junction protein has revealed the presence of gap junctions between these cones. These specialized intercone membrane contacts resemble puncta adherenti that show symmetrical filamentous thickening facing the cytoplasmic compartments of the apposing segments and a wide intermembrane cleft. In some instances, vesicular profiles were found associated with these membrane junctions. In contrast, in the E110 cones, specialized membrane contacts between cone outer segments were not observed after the development of cone outer segments. The transient appearance of specialized membrane contacts between immature cone inner segments suggests a role for these junctions in the emergence of the photoreceptor mosaic.

Supported by EY02593

557.2 DEVELOPMENT OF cGMP-PHOSPHODIESTERASE IN RETINAL PHOTORECEPTORS OF THE RAT, E. Sterret*, and L. Colombani. Istituto di Neurosi, CNR, 56127 Pisa, Italy.

In retinal photoreceptors, the process leading from light absorption to cGMP degradation involves activation of a specific cGMP-phosphodiesterase (cGMP-PDE); this enzyme hydrolyzes 3'-5' cyclic guanosine monophosphate that regulates the permeability of the light-sensitive channels.

We have investigated by immunofluorescence and electron microscopy immunocytochemistry the appearance of cGMP-PDE during the postnatal development of the rat retina. The distribution of cGMP-PDE during development has been compared with that of the adult retina. A monoclonal antibody, ROD1, specific for cGMP-PDE, has been employed. We show that a sudden increase in immunoreactivity takes place during postnatal day 5, when rod outer segments begin to form; immunoreactivity develops rapidly in the following days. Labelling is restricted to the developing photoreceptor outer segments, sparing other cellular districts and other retinal cells, as confirmed by electron microscopy, cGMP-PDE immunoreactivity mirrors the centro-periphery gradient of photoreceptor differentiation.

cGMP-PDE appears in photoreceptor outer segments concomitantly with photoactive rhodopsin and rhodopsin kinase, suggesting a coordination in the appearance of phototransduction proteins during development. If photoreceptor outer segments are provided with their enzymatic machinery, it is conceivable that light dependent electrical events could take place in photoreceptors before the retinal circuitry has completely developed.

557.3 REGULATION OF INTEGRIN ALPHA AND BETA mRNA'S IN DEVELOPING CHICK RETINA. D. B. Getvin, G. M. Cann, A. D. Bradshaw, R. C. Cashwell, C. J. Cummings, A. W. Hunter, R. B. Lebel, E. S. L. Ohl, and W. B. Marshall. National Institute of General Medical Sciences, Baltimore, Maryland 21224, and Division of Molecular and Cellular Biology, Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

Integrins, which are transmembrane proteins that function as receptors for extracellular matrix proteins, appear to be widely distributed in the eye. Recent evidence suggests that the extracellular matrix plays roles in retinoblast migration or translocation, and that matrix responses include those in the integrin family, but little is known about the regulation of integrin expression.

In order to examine the regulation of alpha and beta subunit mRNA's in the developing chick retina, we have attempted to identify integrin mRNA's that are expressed in the developing chick retina. Using a 5'-end labeled probe, 32,000 cDNA clones were screened. Of these, 65 alpha subunit mRNA's and 3 beta subunit mRNA's that are expressed in retinal tissue: Alpha's 2, 4, 6, 8, and v; and beta's 1, 3, and 5. PCR was also used to quantify changes in the relative amounts of these messages during development. We have found that alpha 6 and 8 mRNA levels decrease between embryonic day 6 to embryonic day 9, which correlates with changes in matrix responses that have been observed in cultured embryonic neurons. We speculate that changes in integrin expression may be important for retinal development, particularly during axonal pathfinding.


The tissue distribution of m-calpain (calpain I) and μ-calpain (calpain II) in the eye of the rabbit was examined using polyclonal and monoclonal antibodies against the corresponding rabbit calpains. Purification of the antigens was made as described previously (e.g. Nilsson, et al., Neurobiology of aging, 11 (1990) pp. 425-431) and purified antigens were injected into chickens to produce IgY antibodies recovered from the egg yolk. Monoclonal antibodies were a generous gift of Dr. S. Kawashima. Adult New Zealand White rabbits were perfused fixed with 4% paraformaldehyde and the eyes postfixed overnight after excision. 10 μm thick cryostat sections were cut, blocked with normal rabbit serum and incubated overnight with appropriately diluted primary antibodies. Bound antibodies were visualized with FITC-conjugated rabbit anti-chicken IgG fraction antigodies and sections mounted in DABCO before viewing in a Nikon Microphot FX epi-fluorescence microscope equipped with FITC optics. Distinct immunoreactivity was observed in the embryonic retina on the external and internal surface of the cornea as well as in the epithelial cells covering the iris and ciliary body. The sclera and choroid layers showed intermediate signal staining in the form of varicose fiber-like structures. The immunoreactivity was predominantly associated with areas expected to have a high degree of metabolic activity and/or membrane turnover, in line with the notion that calpains are protein degrading enzymes representing part of a non-lysosomal catalytic pathway. This study supported by the Swedish Medical Research Council, project #03157.

557.5 TRK PROTEIN IS DISTRIBUTED IN A DORSOVENTRAL GRADIENT IN THE EMBRYONIC RAT RETINA. B. H. Fryer,* D. E. Kaplan, W. L. Keifer, and L. E. Bird. Georgetown University Medical Center, Washington, DC 20007 and the National Cancer Institute, Frederick, MD 21702.

During embryonic development, retinal ganglion cells form specific topographic projections in the LGN and tectum. Although it is uncertain what governs this specificity, temporal and axonal gradients of specific molecules on retinal neurons and axons are thought to be important for this process. Since retinal neurons respond to trophic molecules, such as NGF and BDNF, the objective of the present study was to determine whether receptors for these neurotrophins, which belong to the trk family of tyrosine kinase receptors, might also be associated with the appearance of gradients within the embryonic retina. For these experiments immunofluorescence techniques with a pan-trk antibody that recognizes a highly conserved intracellular domain present in all the members used to examine the timcourse of expression of these neurotrophin receptors. By embryonic day 14 (E14), all the cells in the dorsal most retina and their associated axons were present. By embryonic day 18 (E18) and 20 (E20), the DRG was totally labeled and the ventral retina was not stained. These results indicate that during a critical period in retinal development, there is a gradient of trk receptors on retinal cells and their axons which might be involved in the formation of the retinotopic map.


Polyclonal and monoclonal antibodies to the α subunit of the voltage-gated sodium channel (αNaVSD) were used to examine the distribution of sodium channel-like immunoreactivity during the prenatal development of the cat and monkey retina. At all prenatal stages studied, beginning embryonic day 29 (E29) in the cat and E45 in the monkey, both antibodies labeled optic axons. With the polyclonal antibodies the appearance of positive cells largely mirrored the onset of their morphological maturation. For example, αNaVSD immunoreactivity was first observed in the first somata of ganglion cells. A few weeks later horizontal cells displayed immunolabeling. This was followed by immunolabeling of the bipolar cells, the bipolar cell bodies labeling only those cells with the monoclonal antibody some cells were found to be immunoreactive while their somata were still in the ventricular layer (E39 in cat and E52 in monkey). Many of these cells appeared to migrate to the outer portion of the prospective inner nuclear layer where they gradually acquired the morphological appearance of bipolar cells. These results indicate that expression of these sodium channel-like proteins are expressed in the mammalian retina at distinct developmental periods. They also suggest that these proteins could be playing developmental roles unrelated to their function at maturity. Supported by NIH and NMSS.
557.7 LIGHT-INDUCED EXPRESSION OF IMMEDIATE EARLY GENES IN RAT RETINA
Department of Pharmacology and Psychotherapy and Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210.

Induction of immediate-early genes expression occurs in response to a wide range of extracellular stimuli. We present evidence for the expression of c-fos and c-jun in rat retina after exposure to room light. Male Sprague-Dawley rats were maintained in a 12:12 light/dark cycle with lights on at 0800 h. Animals were decapitated either in the dark or after various time intervals in room light. Total RNA from retina was extracted and c-fos, c-jun and NGFI-A mRNA were assayed by Northern blot hybridization. A dramatic increase of c-fos expression was observed in retina of light exposed rats which reached a peak (682%) after 30 min. In contrast, NGFI-A reached a peak after 15 min (246%) of light. Both mRNAs returned to basal values after 2 h of continuous light. Under our lighting conditions, we were unable to observe enhanced expression of c-jun in the retina. Basal content of c-fos, NGFI-A and c-jun were assayed at 0700, 1 h before the onset of room lighting. Injection of MK-801 (NMDA receptor antagonist) or SCH 23390 (D1 dopamine receptor antagonist) 30 min before exposure to light did not prevent the induction of either c-fos or NGFI-A in retina. Our results show that light transiently induces the expression of some immediate early genes in rat retina and that the induction is probably not mediated by glutamate or dopamine receptors.

University of Tennessee, College of Medicine, Memphis, TN 38163.

We are interested in the clone and polyclonal architecture of the mammalian retina. Murine intraspecies chimeras were generated by fusing embryos from ICR and globin transgenic (GT) strains. GT cells were labeled with DNA probes in 20-30 µm from GT-labeling sites in inner and outer nuclear layers. In many instances, GT ganglion cells were displaced horizontally by as much as 20-30 µm from GT-labeling sites in inner and outer nuclear layers. Conversely, unlabeled ganglion cells are often found directly beneath cohorts of GT cells in the nuclear layers. This demonstrates that ganglion cells are often separated from the labeled members of their clones and suggests that these laterally-displaced cells make functional contacts with cells that have different clonal origins, and even different genotypes. Supported by NEI R01-EY8868.

557.9 DYNAMICS OF MOUSE RETINOGENESIS: A POPULATION AND CLONAL ANALYSIS. Dan Goldowitz* and Robert W Williams. University of Tennessee, College of Medicine, Memphis, TN 38163.

We have studied the dynamics of the increase (or net growth) and clone structure in mouse retina. Estimates of the total number of retinal neuroepithelial cells were made in mouse mice (albino ICR strain) at embryonic days E12, E13, E15, the day of birth, and in adults. The retina consists of about 10,000 neuroepithelial cells at E12. There are essentially no postmitotic cells in retina at this age. By E13 the total cell population is ~40,000. By E15, the total cell population has increased to ~100,000, and at maturity the retina contains 3 to 4 million cells. To examine how this 300-fold increase between E12 and E15 is correlated with the development of the clonal and architectural layer of the retina, intraspecies chimeras were generated by fusing embryos from ICR and globin transgenic (GT) strains. GT cells were labeled with DNA probes in E11 or E12 embryos. In retinas of RCS dystrophic rats were immunostained with the monoclonal antibodies RET-P1 and RET-P2. Clones and polyclones in the adult retinas of RCS dystrophic rats were identified by the presence of a variable percentage of cells to chimeric retinas (0.5% to 70%). Clones and polyclones of GT cells are aligned radially and uniformly across inner and outer nuclear layers. In RCS dystrophic retinas, this staining pattern persisted until 2 years in RCS dystrophic rats. Two monoclonal antibodies (RET-P1 and RET-P2) which are specific for RET-P1 and RET-P2 are also being studied in RPE-transplanted RCS retinas.

557.10 DEVELOPMENTAL EXPRESSION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) mRNA IN THE RAT RETINA. G. Casini*, M. Molnar and N. Brecha, Depts. of Anatomy & Cell Biology and Medicine, UCLA and VAMC-West Los Angeles, Los Angeles, CA 90073.

Using in situ hybridization histochemistry, we examined the developmental regulation of VIP mRNA in the rat retina. Retinas collected from birth to postnatal day (PND) 30 were hybridized with a rat VIP RNA probe (from Dr. Goodman) as whole mounts, and then cut into 80 µm parallel to the retinal surface. In adult retinas, VIP mRNA is localized to somata positioned in the proximal inner nuclear layer (INL) and the inner plexiform layer (IPL) of normal retinas and ganglion cell layer. These cells are displaced amacrine cells. Neurons expressing VIP mRNA are sparsely distributed, with a non-random distribution and densities typical of wide-field amacrine cells. VIP mRNA in healthy retina is first detected in the retina during the first postnatal week. The hybridization signal is weak, suggesting low levels of VIP mRNA in these cells. As in the adult retina, labeled neurons are located in the INL, suggesting that these cells express their transmitter phenotype when they are in their final laminar position in the INL. Stronger hybridization and more numerous VIP mRNA-containing neurons are present in the retina at PND 10, which is near eye opening. By PND 15 to 20, the maturation of this cell population is virtually complete. These results show that 1) VIP is expressed in a specific population of amacrine cells in the rat retina; and 2) the developmental expression of VIP is similar to that of other amacrine cell neurotransmitters. Supported by EY04067 and VA Medical Research Funds.


This study was undertaken to further characterize the photoreceptor cell (PRC) rescue effect of RPE-cell transplants to retinas of RCS dystrophic mice (Li and Turner, 1988). Retinas of control and RCS dystrophic mice were immunolabeled with the monoclonal antibodies 8-1P1 and 8-1P2 (anti-human transferrin) and 8-1P4 (anti-human vitamin A-binding protein). There were no differences found between control and RCS dystrophic retinas in transferrin or vitamin A-binding protein immunoreactivity. However, in RCS dystrophic retinas, diminished immunoactivity was noted in the inner nuclear layer (INL) and ganglion cell bodies and their axons in the inner plexiform layer (IPL) of normal retinas. In RCS dystrophic retinas, a similar pattern of PRC bodies and polyclonal architecture of the inner nuclear layer (INL) and ganglion cell bodies and their axons in the inner plexiform layer (IPL) of normal retinas. In RCS dystrophic retinas, a similar pattern of PRC bodies and polyclonal architecture of the retina, intraspecies chimeras were generated by fusing embryos from ICR and globin transgenic (GT) strains. GT cells were labeled with DNA probes in 20-30 µm from GT-labeling sites in inner and outer nuclear layers. In many instances, GT ganglion cells were displaced horizontally by as much as 20-30 µm from GT-labeling sites in inner and outer nuclear layers. Conversely, unlabeled ganglion cells are often found directly beneath cohorts of GT cells in the nuclear layers. This demonstrates that ganglion cells are often separated from the labeled members of their clones and suggests that these laterally-displaced cells make functional contacts with cells that have different clonal origins, and even different genotypes. Supported by NEI R01-EY8868.

557.12 PRENATAL DEVELOPMENT OF HORIZONTAL CELLS IN THE RETINA OF THE RHEUSUS MONKEY. S.J. Kim and M.A. Kirby*. Department of Pediatrics, Loma Linda University, Loma Linda, CA 92350.

Recent studies have shown that the formation of the primate fovea is characterized by the lateral migration of cell classes to present near the fovea. These results suggest that each of these movements is an independent event not directly influenced by the presence of the other. We have compared the number of dendritic terminal clusters in different cell classes to form the fovea.

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THURSDAY PM
557.13
DISPLACED RETINAL GANGLION CELL TOPOGRAPHY SUGGESTS A PROGRESSIVE DEGRADATION OF RETINAL HISTOGENESIS WITH CONTINUED RETINAL GROWTH. A. D. Springer*, Department of Cell Biology and Anatomy, New York Medical College, Valhalla, NY 10595.

Displaced retinal ganglion cells (dRGCs) in goldfish retina were backfilled by applying cobalamin-lysine to the severed optic nerve. dRGCs in flatmounts were defined as labeled cells that were not in focus with the orthotopic RGCs. In sectioned retina, dRGCs were defined as nuclei touching the inner limiting membrane. The x-y coordinates of dRGCs were determined in retina having areas of 11 mm² ≥ 49 mm².

dRGCs represented a nonhomogeneous RGC population since, in large retina, they varied in soma area from 20 - 300 µm². However, the dendritic arbors of these cells were homogeneous in that they were consistently located in the outermost part of the inner plexiform layer. dRGCs represented 0.09% of the total RGCs in small retina and 0.2% of the total RGCs in large retina. This four-fold increase in the percentage of cells that were dRGCs was related to a progressive increase in dRGC density with retinal eccentricity and retinal area. dRGCs were not observed within a 250 µm radius of the optic disc, and their density progressively increased to about 4 cells/mm² at a distance of 3.5 - 4 mm from the optic disc. dRGC density did not differ as a function of retinal quadrant. Sectioned material showed a similar dRGC distribution. Furthermore, doubly-displaced RGCs were found near the outer plexiform layer in the retinal periphery.

The non uniform distribution of dRGCs suggests that repeated retinal ganglion cell division at the retinal margin may be associated with a general, progressive degradation in retinal topography, as well as an increased incidence of ectopic retinal cells. Supported by grant EY-03552 from the NEI.

557.14

An antibody to choline acetyltransferase (AChE) was applied to small (20 mm²) and large (49 mm²) flatmounted retinas. Two different sizes of cholinergic amacrine cells were found in the inner nuclear (INL) and ganglion cell layers (GCL). These cells had a mean soma area of 15 µm² and 50 µm² in the INL and 50 µm² and 65 µm² in the GCL.

Density of large displaced amacrine cells (dAC) decreased from 3.6 mm² in small, to 2.5 mm² in large retina. For small dACs, the density decreased from 1126.4 mm² in small, to 619.5 mm² in large retina. Similarly, small and large orthotopic cholinergic amacrine cell densities in the INL decreased as a function of retinal expansion. Cell density and retinal area were used to estimate the total number of cells per retina.

Concomitant with a 2.5 fold increase in retinal area, the total number of orthotopic cholinergic amacrine cells and small dACs per retina increased by 25%. However, the number of large dACs increased by 71%. The nondisplaced cells in the INL, and the small dACs in the GCL were uniformly distributed across the retina. However, the density of large displaced amacrine cells progressively increased with eccentricity. This increased cell density was unrelated to that normally seen close to the germinal zone.

The paradoxical increase in the occurrence of large dACs as the retina grows may reflect a small degradation in histogenesis with continued production of new retinal cells. Furthermore, large dACs, because of their low incidence (0.2% of all cholinergic dACs), may be ectopic cells. Supported by grant EY-03552 from the NEI.

557.15

A fundamental feature of the retina is the planar mosaic of each neuronal type. We have determined the mosaic of regenerated dopaminergic interplexiform cells, and determined if the absence of the extant dopaminergic cells during regeneration modulated its density (see Reh and Tully, 1986 Dev. Biol., 114:463). Regeneration was induced locally by removing a 0.2-2.0 mm² piece of retina, which was replaced during the subsequent 6-8 days. Two groups of eyes were studied: Group 1 (n=14) received surgical lesions; Group 2 (n=18) received intracocular injections of AChE, to destroy extant dopaminergic cells, 3 wks. prior to the surgical lesion. All retinas were immunostained with antibodies against tyrosine hydroxylase, and regenerated and normal cells were analysed in whole mounts. The data from both groups were statistically identical: the somata of the regenerated TH-cells were more randomly arrayed than normal, and their planimetric density was significantly greater than control values. These data show that regeneration creates a less orderly inner retina than does de novo development, and suggest that, unlike retinal development, extant neurons do not modulate cell proliferation during retinal regeneration. Supported by NIH (NEI) grant EY07060 and EY07063 (CORE).

557.16
VISUAL FUNCTION IN REGENERATING TELEOST RETINA FOLLOWING RETINOCYSTOMY AND CYTOTOXIC LESIONING. A. E. Messinger*, and M. K. Powers Vanderbilt Univ., Nashville, TN.

Components of the electroretinogram (ERG) were recorded in intact fish during regeneration of the neural retina. Portions of the goldfish retina were removed following retinectomy. Additional retinas were destroyed by intracocular injections of ouabain. Contra lateral eyes served as controls.

Following retinectomy, removal of small retinal patches (10-25% retinal area, n=12), b-wave amplitude of experimental eyes increased from 70% (day 14) to 91% (day 75) of control eyes. Experimental eyes with large percentages (75-90%; n=8) of retina aspirated increased b-wave amplitude from 11% (day 14) to 30% (day 75). Complete aspiration (n=9) and ouabain injection (n=37) temporally eliminated ERGs. Following complete aspiration, experimental eyes attained 3% of control b-wave amplitude by day 55. Ouabain treated eyes were 5-10% of controls at day 55 and increased steadily through day 210, when they were 50% of controls. The experiments show that regenerating goldfish retina is responsive to photic stimulation. Partial retinectomy leads to swifter recovery presumably due to a larger population of undamaged retinal cells that can initiate repair. Histological examination of ouabain eyes revealed an occluded lens and a disorganized inner nuclear layer; this probably accounts for experimental eyes only attaining half of control values. Retinectomy circumvents optical side effects and enables a more precise evaluation of the visual sensitivity of the regenerating retina.

557.17

Retinopathy of prematurity (ROP) can be produced by exposure of newborn animals to normobaric hyperoxia (NH) of 60-80% oxygen for several days or several weeks. The proposed mechanisms for ROP are either retinal damage by reactive oxygen species (ROS) or a maintained retinal vasocostriction after NH exposure. We have demonstrated that, unlike adult rat, the newborn rat can survive up to 3 h at 5 atm absolute (ATA) oxygen without mortality or visible morbidity. In the adult rat, however, HBO produces both vasocostriction and ROS before the onset of CHB oxygen toxicity. Because of sensitivity of premature retina to NH, we hypothesized that exposure of newborn rats to acute HBO may accelerate the rate of production of ROS and/or create a severe retinal vasocostriction, which may later result in the development of ROP. Four days postnatal litters (9-11 rats each) were subjected to a single exposure at 5 ATA oxygen for either 30, 60, or 90 min. Histopathological evaluation by light microscopy up to two weeks after HBO exposure showed no detectable retinal damage, unless indicated. In contrast to ROP developed following exposure to NH, the retina of the newborn rat is resistant to HBO. Whether retinal resistance is due to absence of an efficient antioxidant mechanism or because of a reversible but protective retinal vasocostriction, remains to be clarified. Supported by NEI grants No. EY05741 & EY02577.

The early progress of nerve regeneration was studied in sciatric nerve fresh vs. predegenerated autografts in primary vs. delayed nerve repair. Results from the sensory pinch test on alternate postoperative days showed that the 10 mm grafts predegenerated for one week by transaction/ligation and placed in a fresh contralateral host site were superior to fresh nerve grafts. The initial delay period was reduced from 3.6 to 0.2 days, there were no "failures" (0/40 vs. 3/44). The regeneration rate was slightly increased (1.8 vs. 1.5 mm/day) and there was less individual variability. The presence of regenerating axons in the graft was confirmed by immunoocytochemical staining for neurofilament protein. The response of the fresh graft was identical in either a primary or delayed nerve repair. Predegenerated grafts used in delayed repair were better than fresh grafts, but inferior to predegenerated grafts for primary repair. It is suggested that an increased number of Schwann cells is concomitant to more immediate and improved nerve regeneration.


In our companion study of rat sciatic nerve grafts, predegenerated by transaction/ligation for 7 days improved the regeneration compared to fresh nerve grafts. In the present study we determined whether predegeneration for 3 day periods and prepared by nerve crush had a similar effect. Results from the sensory pinch test at 2-10 days showed a nearly identical response for grafts predegenerated for 7 days prepared by either transaction or crush; both regeneration rates were 1.8 mm/day. The efficiency of regenerating axons was confirmed by immunocytochemical staining for neurofilament protein. The regeneration distance at 8 dpo was not significantly affected by the duration of the predegeneration (7-28 days) or the type of injury. These data suggest that predegenerated and newly regenerated axons in the graft prederegenerated by nerve crush do not interfere with the enhancement effect; predegenerated nerve grafts were vated Schwann cells. The predegeneration enhancement effect can be produced by both types of lesions and can last for at least 28 days.

558.3 PRELABELING OF NEURONAL POOLS TO STUDY ACCURACY OF MOTOR AND SENSORY AXONAL REGENERATION IN THE RAT FEMORAL NERVE. B.D. Madigan1, S. A. Bachewich2, T.M. Brushart3, and S.M. Meadows4. Dept. of Surgery (Neurosurgery)1, Neurobiology2, Duke University Medical Center, and Research Service1, VA Hosp., Durham, NC 27710, and Deps. of Neurology and Orthopedics1, Johns Hopkins, and Curtis Hand Center, Baltimore, MD 21218.

Madison and colleagues have recently devised a method of double labeling neuronal pools to determine the accuracy of axon regeneration at the single neuron level (J. Neurosci., Meth., 39, 123-129, 1991). The rat femoral nerve divides into a terminal sensory branch (saphenous nerve) and a terminal motor branch to the quadriceps muscle. Motor axons preferentially regenerate into the terminal motor branch following nerve transection and repair proximal to the terminal bifurcation (Brushart, J. Neurosci., 12, 1026-1031, 1991). In the present study, adult rats received transaction of the femoral nerve either 1 (low) or 4 (high) cm proximal to the terminal bifurcation (N=10 in each group) two weeks prior to labeling of the neuronal pools which contribute to the motor branch (see ref. above). Four weeks later we determined the number of neurons in the original pools which correctly regenerated an axon into the motor branch by using a second fluorescent label and quantifying double-labeled neurons. Approximately 78% and 61% of the motor neurons were double labeled in the low and high transaction groups respectively. Approximately 73% and 49% of the sensory neurons to the muscle spindle fibers were double labeled in the low and high transaction groups respectively. These data show that preferential motor reinnervation takes place regardless of the level of transection, but preferential sensory reinnervation only takes place with a low transaction.

558.4 DISTINCTIVE ABNORMALITIES IN REGENERATED RAT CUTANEOUS AND MUSCLE NERVES AFTER NERVE CRUSH INJURY. C.M. Bone*, N.H. Evans1 and C. Hildbrand2. Dept. of Clinical Neurosciences, Brown Univ., Providence, RI 02912 and Dept. of Cell Biology, Univ. of Linkoping, Linkoping, Sweden.

Regenerated sciatic nerves chronically exhibited a pronounced sensitivity to potassium channel blockade with 4-amino pyridine (4-AP). This has been attributed to the "unnerving" of paranodal potassium channels by myelin sheath remodelling which occurs in regenerated axons. It is not known if these axonal abnormalities are present in all regenerated fibers or if they are restricted to a subpopulation of regenerated axons. The present study examined the morphological and physiological properties of regenerated, myelinated axons in a rat cutaneous and muscle nerve, following unilateral, sciatic nerve crush injury. In vitro, whole nerve recordings (CAPs) were performed in rats with ischemic (R-SN) and lateral gastrocnemius (R-GN) nerves during superfusion with oxygenated Krebs' solution (NS) and during exposure to NS containing 1 mm 4-AP. Light and electron microscopic techniques were used to determine the morphological characteristics of R-SN and R-GN. During recording in NS, latencies to peak CAP amplitude in R-SN and R-LN were comparable to values for their respective control (C) nerves. After 4-AP, a delayed depolarization was noted in R-GN, C-GN and C-SN. In contrast, a pronounced, second negative with a "tripled" appearance was seen in R-SN exposed to 4-AP and recovery properties were significantly compromised. Decreased internodal distances and axonal diameters were observed in R-SN and R-GN but active myelin sheath remodelling was not prominent in either nerve.


Using neuronal and other markers, events of neurogenesis and axon outgrowth in the cricket cercus, a caudal sensory appendage, have been determined to investigate sensory pathway formation during both embryonic development and postembryonic regeneration. In the embryonic cercus, a cluster of neurons first appears near the base of the cercal lumen. These cells project axons to the first connection with the central nervous system (CNS) (1). Subsequently, a second cluster of neurons appears more distally in the lumen and projects axons toward the basal cluster. Formation of these distal cells, neurons arise within the cercal epidermis. The first postembryonic regeneration. In the embryonic cercus, a cluster of neurons first appears near the base of the cereal lumen. These cells project axons to establish the first connection with the central nervous system (CNS). Subsequently, a second cluster of neurons appears more distally in the lumen and projects axons toward the basal cluster. Following formation of these distal cells, neurons arise within the cercal epidermis. The early progress of nerve regeneration was studied in sciatic nerve fresh vs. predegenerated autografts in primary vs. delayed nerve repair. Results from the sensory pinch test on alternate postoperative days showed that the 10 mm grafts predegenerated for one week by transaction/ligation and placed in a fresh contralateral host site were superior to fresh nerve grafts. The initial delay period was reduced from 3.6 to 0.2 days, there were no "failures" (0/40 vs. 3/44). The regeneration rate was slightly increased (1.8 vs. 1.5 mm/day) and there was less individual variability. The presence of regenerating axons in the graft was confirmed by immunoocytochemical staining for neurofilament protein. The response of the fresh graft was identical in either a primary or delayed nerve repair. Predegenerated grafts used in delayed repair were better than fresh grafts, but inferior to predegenerated grafts for primary repair. It is suggested that an increased number of Schwann cells is concomitant to more immediate and improved nerve regeneration.

558.6 PATTERN OF AXON REGENERATION FOLLOWING HYPOGLOSSAL NERVE TRANSECTION AND ENTUBULATION REPAIR. C. Timm-Fair*, G. Chen, and E. deSousa. Laboratories of Clinical Physiology and Experimental Neurology, Dept. of Anatomy and Dept. of Histology, University of Sao Paulo, Sao Paulo, Brazil.

The pattern of axon regeneration following unilateral hypoglossal nerve lesion was studied in adult rats. Transected nerves were repaired by entubulation technique with silicone tubes. Three months after the lesion, animals were processed for morphometric analysis. In one series of experiments, both the regenerated cable found within the tubes and the main medial and lateral branches of the hypoglossal nerve were processed for E.M. Quantitative parameters were determined with a computer system (Biographies Inc.) and compared with uninjured control animals. Regenerated hypoglossal nerves showed a higher number of myelinated and unmyelinated axons in the distal nerve branches and regenerated canal of the hypoglossal nerve. Ultrastructural analysis demonstrated a decrease in the mean axon diameters and myelin thicknesses for the regenerated animals compared to the control group. In another series of experiments, HRP was applied to the cut end of the hypoglossal nerve and the regeneration was compared with non-operated animals processed in the same fashion. The results demonstrated that, after nerve lesion and entubulation repair, no significant loss of motor neurons occurred, but hypoglossal nucleus somatomotor pathway was not established. These findings indicate that after hypoglossal nerve transection and regeneration the normal pattern of axon innervation is only partially restored. Supported by grants from FAES/FINEP and CNPq.
558.7
SENSORY REINNERVATION OF THE RAT GLABROUS SKIN BY ORTHOTOPICALLY GRAFTED FETAL ALLOGENEIC AND XENOGENIC DORSAL ROOT GANGLION CELLS. F. Dubovy, C.M. Rosario, T. Carlstedt and M. Almgren. Departments of Anatomy, Medical Faculty, Brno, Czechoslovakia and Karolinska Institute, S-104 01 Stockholm, Sweden.

Sensory reinnervation of the glabrous skin of the hindpaw in adult rats was examined following orthotopic transplantation of fetal rat or mouse dorsal root ganglia (DRG). Sensory corporules were identified by the presence of nonmyelinated fiber tracts in their non-neuronal cells. Nerve fibers were identified with antibodies to neurofilament proteins (RT-97) or growth-associated protein, GAP-43. Eight months after grafting to the L4 and L5 DRG levels (L6 removed at the time of grafting), saphenous nerve cut seven days prior to sacrifice, RT-97+ nerve fibers were present, some of which terminated in sensory corporules. A few fibers displayed GAP-43 immunoreactivity. In xenografted animals, numerous GAP-43+ nerve fibers were present five weeks after grafting (cf. all-transplant procedure). Some of these fibers were associated with mCherry cells of sensory corporules. These findings demonstrate that orthotopically grafted fetal DRG neurons extend axons all the way to the hindpaw and differentiate into terminals of sensory corporules.

558.8
SPROUTING IN PARITAL, NIGROSTRIATAL LESIONED RATS FOLLOWING ADRENOAL MEDULLA AND SCATIC NERVE COGRAFTS. Z. Zhang, M. Brencic, I. Greenamyre, O. Bokhovka and D.M. Gush. Dept of Neurosurgery, University of Rochester, Rochester, NY 14642.

We report regenerative sprouting of dopaminergic fibers in the striatum following co-grafts of adrenergic medulla and scatic nerve in hemiparkinsonian rats. Adult Fischer 344 rats with unilateral 6-OHDA partial lesions were divided into three groups: nongrafted controls, sham-implanted controls and co-graft animals. At 3 days, 14 days, and each time point were sacrificed on 3, 7, 14, 28 days for immunohistochemical studies and 32 rats used for behavioral evaluation were sacrificed at 3 months and another nine animals sacrificed at 28 days were used for autoradiographic quantitation of high affinity dopamine uptake sites (HADUSA) and D2 receptors. The results of autoradiographic analysis showed that there was a significant increase in the TH positive fibers seen at 3 days in both sham and co-grafted animals. However, TH fiber density in sham-grafted animals was significantly declined by 7 days and nearly reduced to background levels by 28 days post transplantation.

In contrast, TH fiber density remained elevated in co-grafted animals with no significant differences seen between recipients in the 3, 7, 14 or 28 day test groups. Additionally, a positive correlation was found between the maintenance of TH positive fibers density and the viability of Schwann cells in co-grafted hosts. The results of amphetamine-induced ipsilateral rotation revealed a 30 to 45% decrease in the co-grafted group and a sharply increased number of rotations in sham-implanted controls over 3 months compared with the baseline. Post mortem autoradiography showed an increase in HADUSA density as well as a decrease in D2 receptors in the co-grafted striata. All results suggest factors produced by the co-grafts influenced the dopaminergic regenerative response. Supported by NIH 2 P40 NS20778.
**558.13** REGENERATION IV
THURSDAY PM

Mechanisms we examined the expression of the MAb CS-56 in the presence of the glycosaminoglycan chondroitin sulphate also examined by GFAP. Early grafts up to 4 weeks had CS grafts for at least one year where it overlapped with distribution. (NS-17468) (BNS88-15133).

3 weeks. Concomittant distribution of astrocytes was staining only at the host interface after which time it intensely expressed and reactive astroglia have disparate interface. In gray matter surprisingly little gliosis was developed within 6/7 neural lobe explants (after 30 days). From these results we conclude that regenerating retinal axons to pass through the graft and terminate in the superficial part of the SC in 4 grafted animals. In other 3 animals the graft was missing and yet some regenerating fibers were found to traverse the cleft of the sectioned BSC. From these results we conclude that the sciatric nerve graft is beneficial for the functional and morphological restoration of the retinocollicular pathway after BSC section.

**558.15** FORMATION OF NEURAL LOBE-LIKE NEUROVASCULAR CONTACT ZONES IN TISSUES GRAFTED INTO THE HYPOTHALAMIC-NEUROPHYSIOLOGICAL TRACT IN RATS. L. Carlthers* and H.-D. Dellmann. Dept. of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

Large neural lobe-like neurovascular contact regions developed within 6/7 neural lobe explants (after 30 days in vitro axons had disappeared, and explants consisted mainly of pitucytes) grafted into contact with neurosecretory axons transacted in the lateral retrochiasmatic hypothalamic area. Much smaller contact contact regions were seen in 4/9 optic nerve grafts and 3/17 sciatric nerve grafts, and none developed in vascular grafts. In neural lobe explants the fine structure of contact regions resembled that of normal neural lobes; i.e., neurosecretory axon terminals associated with pitucytes formed palisades abutting perivascular spaces of capillaries, most of which were fenestrated. In grafts of optic and sciatric nerves, although neurosecretory axon terminals accompanied by astrocytes incompletely invested capillaries, they did not form palisades, and capillaries were non-fenestrated. Moreover, it was not always possible to delineate neurovascular contact regions within optic and sciatric nerve grafts from contact regions that developed in the adjacent hypothalamic neuropil. These results are consonant with our hypothesis that non-neuronal components of neural lobes, most likely pitucytes, promote development of neurovascular contact regions. Supported in part by NSF grant BNS 8919729.

**558.16** DEVELOPMENTAL CHANGES IN THE RESPONSE OF OPTIC TRACT AXONS TO TRANSECTION IN THE HAMSTER: SWITCHING FROM REGENERATION TO RETRACTION. L. L. Castrucci* and G. E. Schneider. Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Following a complete transection of the brachium of the superior colliculus (SC) in Syrian hamsters, optic tract axons can regenerate if the lesion occurs prior to four days after birth (P4). axons from P4 and older animals normally do not regenerate (So et al., Exp. Neurol. 72, 1981). What is the nature of regeneration or retraction in the hamster? Can we determine if the hamsters can differentiate between them? We used the carbocyanine dye Dil to visualize the response of single optic tract axons to transection at P1 and P6, developmental stages permissive and restrictive, respectively, for regeneration. Retinofugal axons were labeled either in vivo by injecting a solution of Dil dissolved in N,N-dimethylformamide into the eye or by injecting a fixed tissue by placing a crystal of Dil in the optic chiasm. Anterograde labeling with HRP was used to determine the rate of growth or retraction of cut axons. After transection on P1, optic tract axons are rapidly lost from the lesion site, sometimes in large numbers; they can extend up to 800-1000m in 24 hr. Axons crossing the region of the gliosis and beyond are mostly unbranched, and have simple growth cones. Some growth cones remain in the gliotic region longer or grow along the axis of the lesion, perpendicular to their normal pathway. Caudally axon growth appears to slow significantly, nine days after the lesion the SC is still not completely reinnervated. By three weeks the processes of optic tract axons can cover the entire SC.

When the transection occurs on P6, retraction of severed axons can be seen within 12 hr. These axons display retraction bulbs or simple growth cones at their tips. Some axons can remain near the gliotic region of the lesion; some become thinner near the lesion site. Thus, within hours, the response of axons transected at P6 is different from those transected at P1. Supported by NIH grants EY01256 and EY02821.

**559.1** CHONDROIDIN SULPHATE AND ASTROGLIAL DISTRIBUTION IN NEURAL TRANSPLANTS AND IMPLANTED GLIOMA. A. Resenstein* and T. Woods. Dept. of Biochemistry, Molecular Biology, George Washington University Medical Center, Washington, D.C. 20037.

The adenine naloxone used to inject the brain produces potential long term cellular and extracellular changes. To determine the presence of the glycosaminoglycan chondroitin sulphate (CS), a molecule involved in axonal growth by inhibiting mechanisms we examined the expression of the MAb CS-56 in neocortical grafts (2 weeks-16 moe) and C6 glioma (3 days-3 weeks). Distribution of astrocytes was also examined by GFAP. Early grafts up to 4 weeks had CS staining only at the host interface after which time it almost entirely disappeared. Gliosis was found around the grafts for at least one year where it overlapped with extracellular glucose transporter immuno-staining. Stab wounds showed similar results. In gliomas, CS stained intensely particularly at the proliferating tumor-brain interface. In gray matter surprisingly little gliosis was observed whereas in white matter GFAP staining was intense. Ultrastructurally, tumor cells supplanted astrocytes at the interface and CS filled the extracellular spaces. There appears to be a transient expression of CS whereas in tumors CS is intensely expressed and reactive astroglia have disparate distribution. (NS-17468) (BNS88-15133).

**559.2** MIGRATION PATTERNS OF TRANPLANTED LABELLED GLIAL CELLS. A. Espinosa*, A. Watabe, M.-S. Zhang and J. de Vellis. UCLA Mental Retardation Research Center, Los Angeles, CA, 90024-1759.

Gial cell plasticity has been investigated in vitro by numerous laboratories. Such plasticity persists when normal cultured glial cells are grafted into perinatal host brains. Fast Blue labeled O2A lineage cells can survive migrate and integrate within normal and abnormal host brains. In the present study we compared the migration patterns of unaffected rat O2A cells to those of C6 glioma cells grafted into normal rat hosts. Migration patterns of FB* C6 cells are extensive throughout the brain. These cells migrate continuously and form clusters (1 week post-labeling), and tumors later on. Cells derived from such tumors migrated out to form new tumors. FB label persists with variable intensity within grafts despite intense proliferation. This model is useful to investigate the differences in cell migration between normal and transformed cells; the early changes in host brain caused by tumor cells, and graft/host interactions; the plasticity of grafted C6 cells vs cultured C6 cells. (Supported by NIH and DOE)
DIFFERENTIAL SEIZURE SUPPRESSANT EFFECTS OF SLOW NORADENALINE-PRODUCING LUCUS COERULEUS AND SUPERIOR CEREBRAL GANGLION GRANULAT CELLS IN KINDLING. M. Kokaia1, M.A. Fuxe2, P. Kokaia3, F. Bengzon1, O. Nilsson1, A. Brundin2 and O. Lindvall1.1 Restorative Neurology Unit, Department of Neurology, University Hospital, S-205 20 Lund, Sweden.2 Department of Medical Research, University of Lund, S-221 85 Lund, Sweden.

The noradrenergic locus coeruleus (LC) system exerts a strong inhibitory influence on kindling development. We have previously shown that the facilitation of kindling caused by lesions of this system can be reversed by pressor-impoverished LC cell suspension grafts. Similar to intrinsic LC neurons, grafted cells increase their release of noradrenaline (NA) in response to seizures. We investigated whether also LC and superior cerebral ganglion (SCG) cells can influence kindling. Rats were given 6-hydroxydopamine (6-OHDA) in the ventricle and were then subjected to a bilateral aperistaltic lumbar sympathectomy. A phasic elevation of blood pressure (BP) was observed in response to kindling. After 1 week, LC and autologous SCG grafts were placed in the FL lesion cavity. Lesioned and intact animals served as control. After 10-12 weeks the animals were subjected to kindling, microdialysis and histological analysis. LC grafts significantly reduced the development of kindling in previously 6-OHDA-lesioned animals, whereas SCG grafts had no effect. Both baseline and seizure-induced NA release was significantly higher in the SCG grafted animals than in the SCG graft-free group. The lack of effects on the development of kindling in SCG grafted animals can be accounted for by more limited graft-derived fiber outgrowth and NA release. However, the present data may also suggest that the seizure suppressant action of NA is dependent on a synaptic release, which can be provided by the LC but not the SCG grafts.

DENDRITIC OUTGROWTH OF TRANSPLANTED DENTATE GRANULE CELLS. D.L. Legendre, B.P. Vieille and J. Wells. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Allotrogenic neonatal granule cells were injected into the infragranular cleavage plane of the rat dentate gyrus, simultaneously lesioning and replacing the host granule cells. The transplanted granule cells were intracellularly labeled with Lucifer Yellow in fixed sections. Normal granule cells were similar to those already described in the literature. At one week post-transplantation, many of the dentrites that had not matured and had grown converse, and the dendritic spines of the transplanted granule cells formed functional synapses. Grafted cells were placed into the hippocampal fissure, the dentrites developed spines and grew preferentially into the molecular layer of the dentate gyrus rather than CA1.

ADULT OLIVARY AXONS ENLARGE THEIR TERMINAL DOMAIN TO INNERVATE EMBRYOTIC PURKINJE CELLS GRAFTED ON THE SURFACE OF THE ADULT RAT CEREBELLUM. P. Strata, F. Rossi, C. Fabbri and T. Borsellino. Dept. of Human Anatomy and Physiology, I-10125 Turin, Italy. SPON: European Neuroscience Association

It is commonly assumed that neuronal loss in the host brain is necessary for the integration of grafted embryonic neurons. We have studied the capability of grafted cells to interact with host elements in an intact adult brain. To this aim solid pieces of cerebellar primordia taken from 14-days-old rat embryos, were placed in the fourth ventricle of adult hosts in close contact with the cerebellar surface, where they developed microcerebellar Purkinje cells (Pcells). Calbindin antibodies were used to label the host and the transplanted Pcells. In the cerebellar cortex, the development of Pcells was visualized by anti-calbindin antibodies. The synaptic connections of grafted Pcells were analyzed by using transplanted grafted Pcells, where their innervated clusters of PCs were analyzed. The results show that grafted Pcells are able to migrate and develop in an adult unlesioned cerebellum and to interact with host elements by eliciting the growth of adult axons. On their part, adult olivary axons, which face an increased target population, are able to enlarge their terminal domain by retaining their adult targets and innervating a number of transplanted cells.


Survival of grafted neural tissue is improved if the graft is placed into a pre-lesioned area of the host. The overall time course and critical factors underlying enhanced survival and synaptic integration of grafts into lesioned hippocampus remains unclear. This study investigated a number of the availability of neurotrophic factors and vacant synaptic sites on the survival and integration of fetal tissue grafts in a kainic acid lesion model. Disassociated fetal hippocampal and cerebellar cell suspensions were labeled with rhodamine dextran amine and stereotaxically implanted into pre-lesioned hosts. Two-months later hippocampal slices containing the grafts were assessed for graft survival, integration, migration and the development of individual cells using anatomical and physiological indices. Compared to normal hosts, grafts in lesioned hosts showed enhanced cellular survival, development, migration of cells from the needle track and synaptic interconnections with the host. Survival was significantly increased in early transplants (at 2-4 or 11-12 days) compared to controls, grafts at 6-7 days and late grafts at 14-16 and 28-33 days post lesion. Late grafts demonstrated equally good synaptic interconnection but only moderate survival and enhanced migration of cells away from the needle track. Grafted cells showed considerable development of synapse and grafted graft region; synaptic potentials could be elicited with host fiber stimulation. The time course of graft survival and integration indicates that host recovery processes leading to enhanced survival may be separate from factors influencing migration and integration of grafted neurons within the host. This finding has important implications for the timing of grafts following a lesion and the evolution of lesion recovery mechanisms in the host. Supported by NINDS Grant RO1 NS29482-01, ADREA and VAMC Merit Review Awards.

INTRACRANIAL TRANSPLANTATION OF HYPOTHALAMIC NEURONS. Hanna Bergman1, George D Prell2 and Ann-Charlotte Granholm1. 1Dept Cell Biology, Univ. of Linköping, Sweden.2Dept Pharmacol., Mount Sinai School of Med. NY, NY 10029 and 3Dept Basic Science, Univ. of Colorado, HSC, Denver, CO 80262.

Many histamine-containing tuberomammillary neurons of the hypothalamic project to the hippocampus through the fornix. Afferenent denervation has been shown to significantly decrease hippocampal levels of histamine. In order to investigate survival growth and birth of grafted containing histaminergic neurons, fetal hypothalamic tissue from day E17 was grafted into lesion cavities two weeks after unilateral lesions of the fornix in adult Sprague-Dawley rats. Hippocampal levels of histamine and its metabolites were determined in lesion-free, lesioned and lesioned-grafted rats. Post-transplant immunohistochemical evaluation showed that all animals contained surviving transplants; furthermore, numerous immunoreactive fibers projected from transplanted tissue to host brain, including the hippocampus. In conclusion, we found that histaminergic neurons will survive intracranial grafting and interveive surrounding host brain. Sponsored by the Swedish MRC, Blanchefor... Find and Magnus Bergvall... Find and NINDS grant NS-20012 to GDP.
559.10 IMMUNE REACTIONS FOLLOWING MYOBLAST TRANSPLANTATION IN DUCHENNE MUSCULAR DYSTROPHY PATIENTS. T. Tremblay, J.P. Huard, L. Roy, R. Bouchard, J.P. Malouin, F. Richard, M. Guay, M. Fortin, Marie-Louise Langlois, P. Neuroscience Laboratory, Enfant-Jésus Hospital, 1401, 181 Street, Quebec (Qc), G1J 1Z4

The discovery of the genetic defect resulting in a protein deletion disease for Duchenne Muscular Dystrophy (DMD) has led to intense investigation about the therapeutic potential of cell based treatment for this disorder. A possible therapy is the transplantation of normal myoblasts into DMD muscle. This would result in fusion of the donor myoblasts with host myoblasts to form hybrid muscle fibers that have the ability to produce dystrophin and incorporate it into the sarcotendons. Our research group has transplanted myoblasts obtained from an MHC compatible donor to nine Duchenne patients. Transplantations were done in the biceps, erector spinae, carpi radialis longus and/or tibialis anterior muscles. No immunosuppressive treatment was used during the follow-up period (8-19 month). No clinical sign of rejection (i.e., fever, swelling or pain) was observed. Biopsies done 1 month after myoblast transplantation showed the presence of dystrophin in an higher percentage of muscle fibers in the myoblast injected muscle than in the contralateral muscle in 7 of the nine patients. Antibodies against the donor myoblasts and/or myoblasts were observed in five patients. In some case these antibodies were capable of fixing the complement and lysing the myotubes in vitro. Treatment of muscle voluntary contraction were observed in only 3 patients. Although some aspect of these experiments are encouraging, they also indicate that myoblast transplantation cannot be accomplished without some immunosuppression even when the donor is immunocompatible.

559.11 VIABILITY, MORPHOLOGY AND IMMUNOGENICITY OF COLD PRESERVED NERVE ALLOGRAFTS. P.J. Evans*, S.E. Mackinnon, J. Wade and R. Midha. Peripheral Nerve Lab., Univ. of Toronto, Toronto, Ontario, Canada, MSS 1A8

PURPOSE: Nerve banks may provide ready access to tissue and ample time for donor tissue processing. Three week stored nerve allografts may be dead and subsequently immunogenic and serve as a connective tissue scaffold for proximal regenerating host axons.

METHODS: Three centimeter AC rat nerve grafts were harvested and stored in 15 ml of Belzer's UWSS solution at 4°C for 22 weeks. Nerves were prepared for standard light and electron microscopy and for laminin immunostaining. Graft cell viability was assessed by DNA or protein synthesis by in situ fluorometric microscopy. The cell distribution pattern was determined. We found that the distribution of injected myoblasts in the host muscle sections varied according to the different ways of injection. A good injection method allowed even distribution of donor myoblasts over the whole muscle cross-section and it increased the chance for the donor cells to fuse with host myofibers. Mosaic myofibers were observed within seven days after cell injection. The highest fusion rates ranged between 65.0-72.2%. These results are used to improve the efficacy of myoblast transfer therapy. (Supported by PHS NS 26185 P40)

MEMBRANE COMPOSITION AND CELL-SURFACE MACROMOLECULES

560.1 PHOSPHORYLATION OF GAP JUNCTION PROTEIN IN C6 GLIOMA CELLS TRANSFECTED WITH CONNEXIN43 cDNA. M. Deakin, J.F. Bechberger and C.C.G. Naus*, Dept. of Anatomy, The University of Western Ontario, London, Canada N6A 5C1

Gap Junctions are transmembrane channels which aid in intercellular communication permitting the transfer of ions and low MW molecules between cells. C6 glioma cells transfected with cDNA encoding connexin43 (cx43) were used to examine the association of phosphorylation of cx43 on intracellular localization. The total, membrane and cytoplasmic fractions of cx43 were isolated by differential centrifugation, and separated using PAGE and Western Blotting. In these cells, the phosphorylated state of cx43 was found principally in the membranous frations while the non-phosphorylated state was found principally in the cytoplasmic fractions. Following treatment in culture with either 10 μM or 100 μM forskolin to increase cAMP production and protein phosphorylation, an increase in the amount of cx43 protein causes a reduction in growth rate. Supported by a grant from the Medical Research Council of Canada.

560.2 TRANSFERENCE OF C6 GLIOMA CELLS WITH CONNEXIN43 UNDER HUMAN METALLOTHIONEIN PROMOTER CONTROL- EXPRESSION, DYE COUPLING, AND GROWTH RATE FOLLOWING INDUCTION. J.F. Bechberger*, D. Zhu, G.M. Kidder and C.C.G. Naus. Dept. of Anatomy & Zoology, Western University, London, Ontario, Canada, N6A 5C1

C6 glioma cells express very low levels of the gap junction protein, connexin43 (cx43), but upon transfection with cx43 cDNA in a pLTR expression vector, the amount of cx43 protein is greatly increased with a resultant increase in dye coupling and reduced cell growth (Zhu et al., 1991). The pLTR expression vector contains a constitutive promoter, thus individual clones could demonstrate mutational or clonal differences not related to the presence of the exogenous cx43 protein. An expression vector containing a modified metallothionein promoter (pM 2.6) has been engineered which has a very low level of basal activity, but an extremely high inducible expression in the presence of Zn2+ (McClellan et al., 1989). The use of this vector has enabled the transfection clones to be analyzed prior to induction for cellular alterations due to the insertion of the vector and/or clonal selection. Cx43 cDNA was inserted into the pM 2.6 plasmid and stable transfectants were isolated. After 12 hours of Zn2+ treatment (100 μM), several clones demonstrated up to a 25 fold induction of cx43 mRNA, as well as an increase in cx43 protein. Dye coupling analysis of these clones demonstrated a significant increase in functional gap junctions. At present, screening utilizing the complement dependent cytotoxicity assay at 16 hours post-induction (CFN) has been used with intracerebral grafts. The optical dissector directly counts cells in a known volume using the formula Nv=V/((P(Ap)(t)), where V is the sampling volume, P is the number of points, Ap is the calibrated area associated with each point (Ap), and the section thickness (t). In addition to providing a useful parameter in itself, graft volume (V) can be combined with Nv to estimate the total number of cells (Na) by the equation Na=V/Nv. Our resulting estimations of absolute graft volume and unbiased total cell number provide a reliable, comparable basis upon which to assess biological significance.
560.4
MUSCLE CADHERIN IS SPECIFICALLY EXPRESSED IN DEVELOPING SKELETAL MUSCLE OF THE MOUSE EMBRYO B. Mooze, F.S. Walab.
Dept. of Experimental Pathology, UNDS, Guy's Hospital, London SE1 9RT, UK.
We have examined, by in situ hybridization, the spatiotemporal pattern of expression of the major muscle cadherin (N-cad) and the neural cadherin (N-cad) during murine embryogenesis. We compared the N-cad staining with that obtained with neural cadherin specific probes applied on serial tissue sections. The probes used were 5'-labelled cDNA riboprobes. We found that at all embryonic ages examined N-cad was expressed in developing skeletal muscle and was absent from all other tissues. The N-cad transcript corresponded to a 145 kD muscle myotome shortly after its formation. However high levels of myogenin transcripts were found in the myotome prior to N-cad at E8.5. The N-cad transcript was also found in muscles derived from the somites surrounding the vertebræ. Cells immigrating into the forelimb buds, from which the limb muscles differentiate, shows this coordinated expression of several other muscle specific genes.
In contrast the N-cad transcript was found in both the neural tube and the early somite. At later embryonic stages the neural distribution of N-cad predominated as levels decreased in muscle. However transcription of both cadherins was down regulated shortly prior to birth which suggests that it plays a role in cell sorting prior to myoblast fusion.

560.5
CHARACTERIZATION OF CDNAS ENCODING HUMAN CONTACTIN
e.D. Berglund and B. Ramsch, La Jolla Cancer Research Foundation, La Jolla, CA 92037.
We have recently reported the isolation and characterization of a membrane glycoprotein, Gpl35, from human brain (Berglund et al., 1991). Amino acid sequence analysis of a 16 amino terminus and of an internal peptide revealed a strong similarity of Gpl35 to chicken contactin/F11 and mouse F3. These glycoproteins belong to the immunoglobulin superfamily of cell adhesion molecules in the nervous system.
We now report the isolation and characterization of cDNAs clones encoding Gpl35. The open reading frame of Gpl35 consists of six immunoglobulin-like domains and four fibronectin type III domains and thus shares structural similarity with chick contactin/F11 and mouse F3. At the amino acid level, the sequence identity with these proteins is 78 and 94 %, respectively. The sequence terminates with a hydrophobic region indicating that Gpl35 is anchored to the membrane through a glycosyl phosphatidylinositol moiety. Surprisingly, we isolated two types of cDNAs that indicate heterogeneity at the amino terminus of the Gpl35 protein. The two cDNAs are identical except for the insertion of a 33 base pair stretch at the 5'-end. The cDNAs therefore encode proteins of different length with differences at their extreme amino termini. The amino terminus of the longer form is identical to the amino terminal sequence of purified Gpl35 (Berglund et al., 1991) and thus matches the corresponding region of G3 with the exception of a few amino acids. In comparison, the shorter form of Gpl35 lacks 11 residues at the amino terminus and is three amino acids shorter than contactin/F11. By Northern analysis, one mRNA species of approximately 6.6 kb is detected in normal human brain. These data confirm that contactin/F11, F3 and Gpl35 are species homologues.

560.6
Myotubes develop in tightly adherent clusters of cells within which myoblasts fuse on the surfaces of primary myotubes to form secondary myotubes. These then separate from the clusters to become independent myotubes. This study describes the temporal and spatial distribution of NCAM and N-cadherin on muscle cells during fission and myotube separation in the chicken hindlimb. Both LM and EM immunocytochemistry reveal that NCAM, and the low and high stacked forms of NCAM are differentially expressed. N-cadherin is preferentially localized on myoblasts and on newly formed myotubes attached to primary myotubes, but not on the surfaces between more mature myotubes. While NCAM is expressed on all cell surfaces, its polysialylated form is restricted to the free surfaces of muscle cells and is not expressed on surfaces apposed to other cells. Biochemical analyses show that a major portion of polysialic acid is carried on NCAM, and that a shift in the predominant expression of 145 kD NCAM during primary myogenesis corresponds to a rapid and transient increase in immunostaining for polysialic acid. When muscles develop in the absence of nerve activity, myotubes fail to separate from clusters normally. This correlates with a delay in the down-regulation of N-cadherin, a failure of NCAM to be polysialylated, and with a failure in the shift from 145 kD to 130 kD NCAM to occur. Since in other systems NCAM promotes and polysialic acid reduces adhesion, the distributions of N-cadherin and polysialylated NCAM during normal development and the perturbations observed in activity blocked muscles implicate N-cadherin down-regulation and polysialylated NCAM up-regulation in the process of myotube separation from clusters, and indicate that this is a nerve activity dependent process. Supported by NSF grant BNS 9109529.

560.7
EFFECTS OF CYTOSKELETAL INHIBITORS ON N2A AND CHO CELL ADHESION TO N-CAM SUBSTRATES. S.D. Storms, D. Yashmip, J.J. Jensen, and B.A. Murray, Dept. of Dev. and Cell Biology and Dev. Biology Center, Univ. of CA, Irvine, CA 92717.
The role of the cytoskeleton in N-CAM mediated adhesion was studied using a quantitative centrifugal removal assay. In order to evaluate the effects of cytoskeletal perturbation on N-CAM dependent strengthening of adhesion, cells were treated for 30 minutes with 1μM colchicine to inhibit microtubule assembly or with 1μM cytochalasin D to inhibit microfilament formation. Following treatment, N2A and CHO cells were centrifuged on immobilized N-CAM substrates and allowed to incubate at 37°C for 10-60 minutes prior to the removal assay. Strengthening of adhesion in both treatments was not statistically different from controls at P<0.05, although the range of strength of adhesion in both treated groups was more variable than the controls. Adhesion of N2A and CHO cells to laminin was not significantly different in colchicine treated or cytochalasin treated cells, but strengthening was significantly slowed by cytochalasin D treatment. This is expected as laminin is bound by receptors in the integrin family which require cytoskeletal elements for efficient microfilaments. These results suggest that N-CAM binding is independent of the cytoskeleton but may be affected indirectly by cytoskeletal mediated effects on cellular events such as flattening or spreading of the cell.
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WITH LAMININ. Martin Grumet* and Gerald M. Edelman. NYU Medical School, Piscataway, NJ 08854.

560.9


Extracellular matrix (ECM) molecules have been implicated in the regulation of neurite adhesion and outgrowth both during development and after injury. We have previously shown that astrocytes are heterogeneous in terms of expression of the extracellular matrix protein tenasin. High tenasin astrocytes have a reduced ability to support neurite outgrowth. In other experiments, bFGF was shown to cause a significant change in the morphology of cultured neonatal rat cerebral cortical astrocytes and also reduced neuronal adhesion to these astrocytes. In this series of experiments, we tested the hypothesis that bFGF could have increased expression of tenasin by the astrocytes. Cultures of purified protoplasmic astrocytes were established from the cerebral cortex of neonatal rats and subcultured into tissue culture flasks coated with polylysine. Basic FGF was added to test cultures at a concentration of 5 ng/ml, a concentration which was previously shown to cause a significant change in astrocyte morphology and neuronal adhesion on these astrocytes. Tenasin levels were evaluated by Western blot analysis of both extracted cells and conditioned media. Tenasin levels began to increase after 24 h and continued to increase throughout 8 days of treatment. The bFGF treatment was discontinued, and the cells were maintained for an additional 8 days in culture. Tenasin levels returned to control values, demonstrating that the bFGF effect is transient. Neuronal adhesion was reduced on bFGF-treated cultures, suggesting that tenasin may be inhibitory to neuronal growth. It is our hypothesis that the action of growth factors during injury may evoke the induction of tenasin on astrocytes, thereby reducing regeneration in the central nervous system.

560.10

CHARACTERIZATION OF FOUR CELL ATTACHMENT SITES IN CYTOTACTIN. A.L. Prieto, C. Andersson-Flosone, K.L. Crossin*. Dept. of Anatomy and Cell Biology, Duke University Medical Center, Durham, NC 27710.

Cytotactin has domains homologous to epidermal growth factor (EGF), fibronectin type III repeats (FN type III) and fibrinogen (Fg). Previous studies indicate that these domains may represent independent functional units.

To search for functions of the various domains, non-overlapping recombinant protein fragments were made that spanned almost the entire molecule. Cell attachment and morphology of fibroblasts, glia, and neurons were examined using several adhesion assays. In contrast to the previous identification of one cell attachment and one counter-adhesive site, at least four non-overlapping sites on the molecule were found to interact with the cell surface. Two sites mediated cell attachment and were located in the proximal FN type III repeats (1-26 in the chicken sequence) and in the Fg domain; two others were located in the EGF region and the distal FN type III repeats (77-132) and were counteradhesive. The quantitation of attachment on these fragments, competition experiments, and differences in adhesion of the different cell types suggest the following model: cell attachment to different sites on cytactin is mediated by different cell receptors and may be responsible for differential cell adhesion to intact cytactin.
Proteoglycans are known to play an important role in the development of the nervous system. We examined the distribution of proteoglycans in the developing chick retina using an Alcan blue staining procedure. Proteoglycan staining was observed at embryonic day 6 (E6) and at all other stages of embryonic development. Photoreceptors, ganglion cells and the nerve fiber layer were most heavily stained. Moderate staining was seen in all other regions. To determine where neurons deposit newly synthesized proteoglycans, isolated retinal neurons were maintained in culture. Secreted gangliosides and the 245 kDa core glycoprotein are the main physiological product, whereas after three weeks the smaller PG becomes predominant and is essentially the only species present in adult brain. Using the semi-quantitative Rf technique, the 245 kDa core glycoprotein is a part of the 245 kDa PG, because all peptides generated from it could also be found in the larger species. The amino acid sequence of the cloned 150 kDa core protein revealed a high degree of homology with versican and aggrecan, based on the presence of two EGF-like domains, a lectin-like domain, and a complement regulatory-like domain in the C-terminal half of the PG. The larger PG has the ability to form aggregates with hyaluronic acid, and peptide and cDNA sequences from this PG revealed the presence of immunoglobulin folds and tandem repeats similar to those present in the hyaluronic acid binding regions of versican and aggrecan which, however, have much longer glycosaminoglycan attachment domains which cannot be accommodated in the 150 kDa core glycoprotein. Northern blots demonstrated that only a single message of 7.5 kb was detected in either 4-day or adult brain, and no message was detectable in liver, kidney, muscle or lung mRNA. Our results therefore indicate that the adult form of this PG is derived in vivo proteolytic processing from the larger species present in early postnatal brain, and that the 1PG is a new and possibly central nervous tissue-specific, member of the versican/aggrecan PG family.

The axonal reticulum in sympathetic neurons is considered to be an extension of the secretory pole of the Golgi apparatus. If so it would likely contain the enzymes involved in catecholamine elaboration.

To test this hypothesis the distribution of DSH and cytochrome b561 was investigated in bovine spenic nerve and nerve terminals in the vas deferens with an immunogold procedure after glycolmethacrylate embedding.

Counterstaining was with phosphotungstic acid at low pH.

With antibodies against both enzymes gold labeling was detected over the large dense-cored vesicles, the Golgi-associated axonal reticulum, the reticulum within axons and over the tubular complex at the nerve terminal.

From our results it can be concluded that in sympathetic neurons the axonal reticulum represents a tubular, neurosecretory transport system, spanning the neuronal cell from Golgi apparatus to nerve terminal.


Sialyltransferases (STs) are a family of glycosyltransferases which catalyze the transfer of sialic acid (NeuAc) to the non-reducing terminal sugar of glycoproteins and glycolipids. One of the key enzymes in the synthesis of gangliosides is CMP-sialic acid: LacCer sialyltransferase (ST-I) which catalyzes the transfer of sialic acid to lactosylceramide (CDH) to form GM1.

In this report we describe the purification and characterization of ST-I from a Triton X-100 extract from rat brain. The enzyme was purified by affinity chromatography on CMP-Sepharose and resolved by NaCl gradient elution from the same adsorbent. Further purification of GM1 synthase was achieved by chromatography on a "CDH-acid"-Sepharose column eluted with CDH. The enzyme activity was highest at pH 6.5 and required Triton CF-54 or Triton X-100 (0.1%) for full activity. The apparent Km value for CMP-sialic acid was 170 μM. These data correspond with the results obtained for ST-I purified from rat liver (1), suggesting that both enzymes may share a common domain structure or may have significant sequence homology. (Supported by USPHS grants NS-11853 and A. von Humboldt Found. to U.P.)


DIFFERENTIAL EXPRESSION OF GLUCOSE TRANSPORTERS, GLUT3 AND GLUT1, IN CULTURED CEREBELLAR GRANULE NEURONS: EFFECTS OF POTASSIUM AND NMDA. Frances Maher* and Ian A. Simpson. NIDDK, NIH. Bethesda, MD 20892.

Cerebellar granule neurons in culture express two glucose transporter isoforms, GLUT3 and GLUT1. GLUT3 is the predominant isoform. We hypothesize that the chronic effects of depolarizing stimuli, i.e. high potassium concentration and NMDA, on glucose transporter expression as determined by immunoblotting with antisera to an epitope common to GLUT3 and GLUT1, would be increased by 20% decline in cell number. In K5 medium, 2-deoxyglucose transport activity was decreased by 50-60%. This corresponded to decreased glucose transporter expression as determined by immunoblotting with antisera to an epitope common to GLUT3 and GLUT1, by 20%. Addition of NMDA (100-200μM) to K5 and K15 medium stimulated GLUT3 expression to the levels in K25 but failed to fully restore GLUT1 levels. The effect of NMDA was blocked by APV. Potassium concentration, NMDA and other EAA had no acute effects on glucose transport or GLUT1 and GLUT3 levels. Expression of Na+/K+ ATPase isoforms was not altered by these culture conditions. Immunoblotting, immunofluorescence and NMDA receptor activation result in optimal expression of neuronal glucose transporters, the GLUT3 isoform being more sensitive than GLUT1 to chronic regulation by these conditions.

THE LOCALIZATION OF A NOVEL CA2+-BINDING PROTEIN (CBP-18) IN THE RAT BRAIN. D.P. Wolfe*, H.P. Lipp, W.X. Qin and Ch. W. Heizmann. Institute of Anatomy, University of Zurich, CH-8057 Zurich, Switzerland.

The distribution of a novel calcium-binding protein (CBP-18) with a molecular weight of 18 KDa in the rat brain (Manalan & Klee, J. Biol. Chemistry 259, 2047-2050, 1984) was studied by means of immunohistochemistry on cryostat-sectioned tissue and compared with staining patterns of parvalbumin on adjacent sections.

The polyclonal rabbit-derived antibody for CBP-18 showed selective affinity for periglomerular cells and dendrites in the olfactory bulb, and also distinctly stained some cells and dendrites in the anterior olfactory nucleus. Marked diffuse pericellular staining of CA1 pyramidal cells and of collateral (including proximal dendrites) was observed in the retrosplenial cortex, hippocampal rudiment, the septum, area preoptica, hypothalamus, in the parabrachial nuclei and in the cerebellar neuropil of both the molecular and the granule cell layer. Less intense neuropil staining for CBP-18 was found in the neocortex, the remaining basal forebrain, parts of the colliculus superior, and in the entire brain stem. Neuronal staining was barely detectable or missing in the striatum, the hippocampus, the thalamus, the colliculi, the reticular formation, the olivary and inferior olive nuclei, the brainstem, and the cerebellum. CBP-18 appeared to stain regularly cross-sectioned axons but rarely longitudinal fibers. Thus, CBP-18 shows an unique staining pattern in the CNS different from all other Ca2+-binding proteins studied so far. Supported by Swiss National Science Foundation for Scientific Research (SNF 31-27737.89, 31-9470 and 31-30742).


The mechanisms of rapid actions of estrogen, such as the rapid release of prolactin from GH3/B6 cells, have not been elucidated. We hypothesize that a membrane form of the estrogen receptor (ER), similar to the intracellular ER may mediate some of these actions. A polyclonal antibody (anti-ER) was generated to a peptide corresponding to amino acids 270-284 of rat ER. The antisera recognizes both native (sucrose density gradient) and denatured ER from rat uterine cytosol, and peptide affinity purified antisera recognizes a single species of 67 KD on immunoblotts. GH3/B6 cells were separated by estrogen affinity chromatography using magnetic beads. When these enriched populations were incubated at 2°C with the affinity purified anti-ER, indirect immunocytochemistry revealed heterogeneous membrane labeling. Anti-Actin controls showed that cells were not permeabilized by the live-labeling procedure. When cells were brought to 37°C after anti-ER incubation, labeling became "patchy" and decreased in amount, disappearing by 15 min. Nuclear and cytoplasmic labeling were also evident in cells permeabilized with detergent after fixation. Both membrane and intracellular labeling could be competed with the peptide used to generate the antibody.


Previously we reported that the epitope defined by the monoclonal antibody Tor 23 is on a presynaptic form of Torpedo acetylcholinesterase (AChE), which associates with the membrane via a GPI linkage. To confirm the conformational architecture of the epitope we have performed a series of Triton X-100 solubilization experiments. Although Triton does not inhibit AChE activity, even trace amounts (0.001%) interfered with the immunoprecipitation of AChE by Tor 23. To test whether the lack of precipitated AChE was due to a change in the AChE molecule such that the antibody failed to recognize it, Triton was added to immune precipitates and the pellet and supernatant assayed for AChE activity. Triton released AChE activity from the pellet into the supernatant. In contrast, AChE activity remained in the pellet in untreated samples. These experiments support other data suggesting that Tor 23 identifies a conformational epitope of presynaptic Torpedo AChE.

Although we have no data that Tor 23 recognizes AChE in mammals, the antibody inhibits high affinity hemicholinium-3 binding, high affinity choline uptake and acetylcholine synthesis in rat neocortex (Evans et al., submitted). Furthermore, our studies of neuroblastoma cell lines using mannosamine suggest that, as in the Torpedo, the Tor 23 antigen has a GPI linkage in systems in which the hypothesis that the Tor 23 antigen, in both Torpedo and mammalian systems, is a GPI-linked molecule involved in cholinergic processing. This work is supported by  the State Estate.
MEMBRANE COMPOSITION AND CELL-SURFACE MACROMOLECULES

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The tyrosine kinase receptor c-brains of adult baboon and rhesus monkey with SR-1 and mouse brains melanoblasts, germ cells and hematopoietic stem cells. Using a mouse-kit ligand, stem cell factor (SCF), is coded by the expression in adult primate and mouse brains.

J.Y. Li

were observed in many areas including olfactory bulb, cerebral cortex, septum. The staining appeared to be on fiber tracts and terminals but inferior olive. In the brainstem and spinal cord, spinal trigeminal nucleus, gracile and cuneate, observed in the hippocampus, dentate gyrus, fimbria-fornix and lateral around Purkinje cell bodies. Staining was also seen in the molecular layer to homogenate consistent with a postsynaptic localization. The results suggest that the isoforms may have different affinities to organelles in fast anterograde transport.

THURSDAY PM


Northern blot analysis of total RNA from rat brain revealed heterogeneity in mRNAs encoding the Na/Ca exchanger. The blots were probed with an 800 bp full-length DNA probe coding for the rat cardiac Na/Ca exchanger and with the full length DNA probe coding for human heart Na/Ca exchanger (Kofuji et al. Biophys. J. 61: A387, 1992). Blots were washed using medium stringency conditions (0.1% x SSC at 37°C). We identified three Na/Ca mRNAs (15, 20 and 3.2 kbp) not only in adult whole rat brain total RNA, but also in the developing rat brain total RNA in late embryonic stage (day 18). Northern blot analysis of total RNA from cultured cortical cells, 10 days in vitro, however, showed only a 7 kbp Na/Ca exchanger mRNA under the same conditions. Immunohistochemical Western blot analysis of the astrocytes, synaptic plasma membranes and heart sarcolemma proteins using polyclonal antibody raised against purified canine heart Na/Ca exchanger (Ambesi et al., Biophys. J. 59: 138a, 1991) revealed the same protein band pattern in all samples: 70, 120 and 140 kDa. These data suggest that the expressed Na/Ca exchanger in whole brain and in astrocytes may be the same protein, highly homologous to human Na/Ca exchanger. The different-sized mRNAs probably vary from each other only in their untranslated regions.

560.30

TYROSINE PHOSPHORYLATION OF GP180 IN ADULT AND DEVELOPING BRAIN. G.M. Gurd, N. Bisson, M. Khurgel and J. Soulliere. Scarborough Campus, University of Toronto, West Hill, Ontario, M3J 1A4

We have reported the presence of protein tyrosine kinases in isoform specific antibodies (Gurd and Bisson, J. Neurosci. Res. 25 336-344). We have now used Western blotting of rat brain homogenates with anti-phosphotyrosine antibodies to determine the distribution of ptyr-containing GP180 (PTGIP180). PTGIP180 was enriched approximately 20-fold in P50s relative to homogenate consistent with a postsynaptic localization. Cerebellum and hippocampus contained low levels (less than 15% of forebrain) of PTGIP180. P50s from these regions contain GP180 suggesting regional variation in the activities of postsynaptic tyrosine kinases or ptyr-phosphatases or both. Forebrain homogenates from newborn rats contained less than 5% of the adult level of PTGIP180. The amount of PTGIP180 increased markedly during the third and fourth weeks of postnatal development before declining to adult values. Incubation of P50s with ATP increased the tyrosine phosphorylation of GP180 to 15-fold, indicating that less than 10% of potential tyrosine phosphorylation sites are occupied in vivo. Supported by the N.S.E.R.C.

560.32


Carotenoid deprivation in Drosophila reduces visual pigment, opsin, size of the rhabdom and P-face particle density; replacement by feeding carrot juice rapidly restores visual pigment (Sapp et al., 1991, Exp Eye Res. 53: 71). Our data indicate that this effect is mediated by retinoid-activated opsin gene transcription (Stark et al. 1992. Invest. Ophthal. Vis. Sci. 33: 1398). Here we report that P-face particle density also increases in rhabdomeric microvilli in the early days of replacement therapy to 3000 particles per day, reaching the control level of over 4000 by day 2. Our data reveal a continuity of the microvilli with the adjacent retinula cell plasmalemma between the microvilli and the rhabdomere. This plasmalemma reflects the rhabdomeric P-face particle density. Freeze-fracture preparations of Drosophila photoreceptors show that microvilli budding from bases of microvilli and from plasmalemma as well as multivesicular bodies and Golgi apparatus. Recovery in Drosophila is considered complete as recovery induced by 11-cis retinal in similarly deprived Manduca (Bennett & White, 1991, Vis. Neurosci. 6: 473). Further, there are substantial differences in the endomembrane traffic in deprivation vs. replacement. Support: NSF BNS8811062 & NIH EY07192 (WSS) & NSF BNS91 10672 (RW).

560.31


The tyrosine kinase receptor c-kt is coded by the W locus and its ligand, stem cell factor (SCF), can play a critical role for the development of neural crest-derived melanoblasts, germ cells and hematopoietic stem cells. Using a mouse monoclonal antibody to c-kt and a rat monoclonal mouse c-kt antibody (ACK2), the expression of c-kt was studied in the brains of adult baboon and rhesus monkey with SR-1 and mouse brains with ACK2 by conventional immunohistochemistry. c-kt IR was observed in the hippocampus, dentate gyrus, fimbria-fornix and lateral septum. The staining appeared to be on fiber tracts and terminals but not on the hippocampal and dentate pyramidal cells. Distinct c-kt IR were observed in many areas including olfactory bulb, cerebral cortex, amygdala, caudate putamen, thalamus, interpeduncular nucleus, and inferior olive. In the brainstem and spinal cord c-kt IR were associated with the sensory pathways which included dorsal horn in the spinal cord, spinal trigeminal nucleus and tract, gracile and cuneate, vestibulocochlear nucleus and solitary tract and its nucleus. In the cerebellum, very intense staining was on Purkinje cell axon hillock and around Purkinje cell bodies. Staining was also seen in the molecular layer and upper granule layer. This study and recent reports on the expression of mRNAs for both c-kt and SCF in mouse brain (Morro et al., Development 113:1207-1211, Mori et al., Dev. Brain Res. 65:123-126) suggest potential functions of these proteins in the nervous system.
561.3
CULTURED POSTMITOTIC NEURONS DO NOT UNDERGO VIREO-MEDIATED HOST CELL SHUTOFF OF PROTEIN SYNTHESIS AFTER INFECTION WITH HERPES SIMPLEX VIRUS-V. A UNIQUE RESPONSE IN NEURONS AND A PROMISING RESULT FOR HSV-1 VECTOR TECHNOLOGY. P. F. Nichols, J. Y. Chang, L. S. Greenlund, P. Olivo, E. M. Johnson, Jr. Washington University School of Medicine, St. Louis, MO 63110

Herpes simplex virus (HSV)-derived vectors or recombinant HSV have been proposed and preliminarily characterized as vehicles for expression of foreign genes in neurons. A potential factor compromising the utility of such vehicles is the ability of HSV to produce virus-induced host cell shutoff (VHS), a phenomenon mediated by a viron-associated protein (UL41). Upon infection, the UL41 gene product decreases protein synthesis (20-50% of controls) by disrupting translation, degrading cellular transcripts, and potentially preventing further cellular transcription. If such a shutoff were to occur in neurons, a significant alteration of the cellular physiology would be produced that might affect the ability of a neuron to express and respond to foreign genes. VHS has not been examined in neurons. We demonstrate that shutdown of global protein synthesis does not occur after infection of cultured postmitotic neurons with HSV. In infected neurons infected with d120 or KOS-derived vhs-A5ma, a deletion mutant that lacks the UL41 gene. Undifferentiated or NGF-differentiated PC-12 cells and Vero cells experienced VHS upon infection with HSV. VHS has not been examined in neurons. We demonstrate that shutdown of global protein synthesis does not occur after infection of cultured rat sympathetic neurons with HSV-1 strains KOS and, the replication incompetent mutant, KOS d120. Also, no difference is seen in the rate of protein synthesis in neurons infected with d120 or KOS-derived vhs-A5ma, a deletion mutant that lacks the UL41 gene. Undifferentiated or NGF-differentiated PC-12 cells and Vero cells experienced VHS upon infection with KOS-derived d120 (a decrease in protein synthesis to 40% of controls at an M.O.I. of 20). The results suggest that neurons are resistant to VHS as mediated by the UL41 gene product. This apparent lack of VHS may be important in the inability of HSV to become latent in these neurons. The results indicate that VHS should not be a complicating factor in these, and perhaps all, postmitotic neurons. (Supported by the American Paralysis Association and by the Monsanto Corporation.)

561.4
CHARACTERIZATION OF ISO- AND DIVERSITY-FORMS OF NCAM IN PRIMARY AND TRANSFECTED CELLS. D. Barthels1, W. H. Hartmann1, A. Christoff2, G. Wenger1, M. Bermingham2, G. Barron2, and W. Willer. Institut für Genetik, Universität zu Köln, FRG, and Centro de Biología Molecular, Universidad Autónoma, Madrid, Spain

Alternative RNA splicing of primary transcripts coding for cell adhesion molecules is the main source for immune-mediated diversity. A well-analyzed example is the neural cell adhesion molecule (NCAM). The Ig-family gene encodes for 92 NCAM amino acid sequences (Barthels et al. 1992). In addition to posttranslational modifications, alternative splicing significantly contributes to the diversity of iso-forms. These domains are important for the function of different NCAMs. Transfection of NCAM-CDNA sequences into murine cells provides a useful test to investigate interactions of specific NCAM forms with primary neurons. For this analysis one needs a sensitive detection system for the influence of different NCAM iso- and diversity-forms on neuronal adhesion and fiber outgrowth. One of the in vitro systems discussed is the modified stripe assay (after F.Bohnhoeffer), in which axon-forming primary neurons are transfected with numerous decisions between alternative substrates. In addition, specific antibodies and monoclonal Abs against diversity epitopes have been developed in order to identify specific gene products and to interfere with defined functions. The second strategy is to investigate the biological relevance of NCAM diversity forms by means of their regulation in defined parts of the CNS. We choose the embryonic chicken retina for this study because the limited number of neuronal cell types and the well defined developmental pattern.
GENE STRUCTURE AND FUNCTION VII

561.5
DIFFERENT STRUCTURAL REQUIREMENTS FOR MAO A AND B CATALYTIC ACTIVITY. H.-W. K. Chen, and J.-C. Shih. Dept. of Mol. Pharm. and Tox., Sch. of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033.

There are seven conserved cysteines in the deduced amino acid sequences of human liver MAO A and MAO B. Site-directed mutagenesis of these cysteines to serine showed that the MAO catalytic activity was totally lost when FAD-linked cysteine was mutated (Cys 268 and Cys 356 for A and B respectively). Mutant Cys-156 and Cys-356 of MAO A and B also lost their activities, but the corresponding MAO A mutant Cys-165 remained active.

Chimeric MAO A/B was generated. When the N-terminal 30 amino acids were exchanged between A and B, the catalytic properties remained the same, suggesting that the N-terminus may not be important for enzyme specificity. When the C-terminus 125 amino acids were exchanged, MAO A with MAO B C-terminus has normal MAO A activity, suggesting the C-terminus of MAO A has no effect on the A activity. However MAO B with MAO A C-terminus is catalytically inactive. This result suggests the C-terminus of MAO B is critical for MAO B activity. Taken together, these results suggest that the tertiary structure for MAO A and B may be different and the requirement for MAO B activity is modified by N-terminal 30 amino acids.

561.6

The dopamine transporter's (DAT) 12 cysteine putative transmembrane domains contain polar amino acid residues that are candidates for involvement in recognizing cocaine analogs (eg. CFT), dopamine (DA) and the dopamine neurotoxin MPP+ (see abstract by Kitayama et al. DAT cDNAs mutated by changing polar amino acids in domains 9 and 12 to alanines were examined in COS cells for their abilities to mediate DA and MPP+ uptakes and CFT binding. Mutants in domains 9 and 12 ablate each function. Mutants in domains 8 and 10 and more selective mutations in domain 12 reduce DA and MPP+ uptakes and CFT binding by 50-80%. Mutations in domain 11 decrease DA and MPP+ uptakes without changing CFT binding.

Differential effects of changes in different domains are consistent with differential contributions of each to DAT function in dopamine transport and cocaine recognition.

561.7

Polar amino acids lying within putative transmembrane (TM) regions 1, 7 and 8 of the dopamine transporter (DAT) are analogous to those important in catecholamine receptors. Their possible functional significance was examined by testing binding and function in COS cells expressing mutant DAT cDNAs. Substitution of aspartate at position 79 in TM 1 with alanine, glycine or glutamate dramatically reduced uptake of [3H] dopamine and [3H] MPP+ and the mutants' affinity for the cocaine analog [3H] CFT without affecting I benefiting. Replacing serine at positions 356 and 359 in the outer portion of TM 7 by alanine or glycine reduced dopamine and MPP+ uptake, while [3H] CFT binding was less affected. Mutations in serine residues predicted to lie in the inner half of TM 7, 356 and 359, yield enhanced dopamine and MPP+ uptake, with little change in [3H] CFT binding I benefiting. Substitution of two residues in TM 8 results in wild-type values for dopamine and MPP+ uptake and [3H] CFT binding.

The TM 13 residue is thus crucial for cocaine binding and dopamine uptake, while serine residues in TM 7 appear to play larger roles in substrate transport. These data define molecular features differentially important for cocaine binding and for dopamine uptake.

561.9

ATP (0.1 mM) and (methyl-3H)-thymidine (20 Ci/mmol) were added to the culture medium, and the incorporated 3H-thymidine into DNA of PC12 cells were measured as an acid insoluble precipitate. We investigated the stimulant-evoked DNA synthesis. Only ATP showed the stimulation of the DNA synthesis in PC12 cells, if the ATP was washed out from medium after the stimulation of cells for 10 min, the DNA synthesis in PC12 cells was totally lost. By the treatment, a protein synthesizes inhibitor, but was completely ineffective. These results suggested that the ATP-evoked DNA synthesis was stimulated through P2 purinergic receptor. This DNA synthesis was insensitive to aphidicoline treatment (0.1 ng/ml), a DNA replication inhibitor, and the growth rate of ATP treated PC12 cells decreased to a half of control cells. Thus, the ATP was not recognized as a mitogenic stimulus for PC12 cells. The ATP-evoked DNA synthesis was insensitive to cycloheximide treatment, a protein synthesizes inhibitor, but was completely ineffective by actinomycin D treatment (0.1 µg/ml), which is a RNA synthesizes inhibitor. These results suggested that the ATP-evoked DNA synthesis occurred through RNA dependent DNA synthesis was observed in the differentiated PC12 cells and in the primary culture of rat hippocampus.

561.8
METHYLATION OF CGG SEQUENCES IN THE GLIAL FIBRILLARY ACIDIC PROTEIN GENE PROMOTER IN RAT ASTROCYTES. B.D. TETER*, H.H. OSTERBERG, C.E. FINCH. Neurogerontology Division, University of Southern California, Los Angeles, CA 90089-0191.

The astrocyte-specific gene encoding glial fibrillary acidic protein (GFAP), an intermediate neurulloinament, increases in expression in the brain with age, as well as with hormone manipulations and deafferenting lesions. Analysis of sequence data (Mura et al., 1990, J. Neuroch. 55:1180) shows that the mouse GFAP gene promoter contains an island of eight CGG sequences (CGG dinucleotides are rare in eukaryotic genomes). Limited sequencing of the rat GFAP promoter shows additional CGG sites; the absence of these sites in the mouse promoter is consistent with their loss by mutation of methyl-cytosine to thymine. CGG sequences are sites of cytosine methylation which is correlated with promoter activity (in general, CGG methylation is positively correlated with activity). Cytosine methylation is associated with a generalized decrease in rat brain with age (Das and Das, 1986, Biochem. Arch. 53:395), which suggests that demethylation of the GFAP promoter CpG island will correlate with the age-related increase in GFAP expression. We are investigating the pattern of CGG methylation in the GFAP promoter in GFAP-expressing tissues (hippocampus) and non-expressing tissues (live) using ligase-mediated polymersize chain reaction (LMPCR). We are characterizing the pattern of CpG methylation in C6 glioma cells and any changes with corticosterone treatment (see abstract by C.J. Huang, et al.). Results will evaluate whether the pattern of GFAP promoter CpG methylation is correlated with constitutive and induced GFAP expression and whether a change in the methylation pattern with age is independent on GFAP expression. Supported by PHS grant AG 00093 and AG 7908.

561.10
In Vitro Pre-mRNA Splicing of the Amyloid Precursor Protein Gene L.C. Raff and G.A. Higgins. Molecular Neurobiology Section, NIA/NIH, Baltimore, MD 21224.

Alzheimer's disease (AD) and Down's syndrome (DS) is characterized by the extracellular deposition of amyloid in the form of senile plaques and cerebrovascular amyloid. The major component of these neuropathological markers is a 4 kDa protein called the 64A protein, which is derived from a larger 120 to 130 KD precursor protein. The amyloid protein precursor (APP) gene contains at least 18 exons, which undergo alternative RNA splicing to produce at least nine different precursors and their products. APP-695 is the primary form found in the brain and lacks two exons (67 and 68), while APP-751 lacks two exons (63 and 64). The APP-770 has been used to study the structure of the APP gene, through alternative splicing, may play a role in the deposition of the 64A peptide found in the senile plaques and cerebrovascular of AD and DS brains. We have developed a model of the APP gene splicing system. Branchpoints are being identified using primer extension analysis of lariat intermediates of the in vitro splicing reactions. A subcloned fragment of a full-length cDNA for APP-770 has been used in RNA splicing analysis to quantify the relative levels of the wild-type APP transcripts normally produced by HeLa cells. Future studies involve development of a cellular model to study splicing of our minigene constructs, and mutagenesis is being used to identify critical cis-acting elements required for correct splice site selection.
561.1
ALTERNATIVE SPlicing OF A HUMAN AMLYOid PRECURSOR PROTEIN MINI-GENE CONSTRUCT IN MOUSE N2A NEURoblastoma CELLS. D. Wolcott*, S. A. Johnson, and C. E. Finch, Andrus Gerontology Center and Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089.

In Alzheimer's disease, the amyloid precursor protein (APP) is cleaved abnormally to produce a 42-43 amino acid amyloid beta peptide that accumulates extracellularly as a major component of senile plaques. Alternatively spliced APP-mRNAs encode 695, 731 and 770 amino acid polypeptides. This alteration in coding potential of APP-mRNAs is conferred by the variable inclusion of exon 7 and exon 8 of the APP gene. Exon 7 encodes a 65 amino acid Kunitz protease inhibitor domain, while exon 8 encodes a 19 amino acid motif with similarity to NGF, a neuron and glial cell cytoskeleton protein. Alternative splicing of APP-mRNAs has been observed in neurons undergoing degeneration and as an in vivo neuronal response to neurotoxic lesions. We constructed a plasmid pScy6-7-9 which lacks exon 8, gave rise to splice variants both containing and lacking exon 7. Alternatively spliced mRNAs derived from pScy6-7 were also detected in transfected rat C6 glioma cells. pScy6-9 in transfected neuroblastoma cells can be used to explore the mechanisms of APP mRNA differential expression during neuronal differentiation and in response to neuronal trauma. DW was supported by TO-AG0037-13; this work was supported by AG1909 to C.E.F.

561.12
SYNTHESIS OF cRNA PROBES FROM PCR-GENERATED MINGENES. J. Logel*, D. Dill, C. Drebing, S. Leonard, Denver Veterans Administration Medical Center, Denver, CO 80220.

Many molecular biological techniques such as northern, RNA protection and in situ hybridization analyses are dependent on the availability of cloned cDNAs in vectors containing T7, T3 and SP6 RNA promoter sequences. We report a method for in vitro transcription of cRNA probes using PCR generated DNA fragments or minigenes. Sense oligonucleotide primers, specific for mouse acidic fibroblast growth factor (α-NGF), were prepared in vitro and served as templates for primer extension reactions. These cDNA templates were synthesized with 5′-extensions containing sequences for the T7, T3 and SP6 polymerase promoters. A common antisense primer was used with each of the promoter/α-NGF primers to prepare PCR-generated DNA fragments (mingenesis). In vitro transcription efficiency for each of these constructs was evaluated by incorporation of radioactive energy into the cRNA products. We find that both the T7 and T3 promoters can direct the synthesis of cRNA probes of high specificity from a PCR-generated DNA fragment, but the SP6 promoter cannot. Antisense cRNA probes, transcribed from minigene constructs for α-NGF were used for in vivo and in vitro transcription of minigene constructs for β-nerve growth factor (β-NGF) were used to synthesize antisense and sense cRNA probes for in vivo hybridization of human hippocampal tissue sections.

GENE STRUCTURE AND FUNCTION VII THURSDAY PM

562.1

Following nerve injury, axonotomized neurons respond by reducing the number of genes associated with neuronal growth, and Schwann cells distal to the site of injury down-regulate genes associated with myelination. We hypothesized that at least some of these alterations were due to the loss of ongoing homeostatic signals that were transduced as a function of fast axonal transport. To directly address this hypothesis, we selectively blocked fast axonal transport in vivo by locally-cooling nerves to 3-4°C (a cold block). Immunocytochemistry for the antigens OX-42 or ED1 demonstrated that macrophages do not accumulate around the cold block. Furthermore, neurons rapidly regained the ability to retrogradely transport tracers upon removal of the cold block. Thus, any effects of the cold block were not likely due to nerve injury. To determine whether blocking fast axonal transport was sufficient to induce a neuronal "atony" response, we examined expression of Tα-1, Tβ-1 and p75 NGF receptor mRNAs in facial motoneurons. In situ hybridization and image analysis revealed that both of these mRNAs were induced to a similar degree 36-60 hrs following either a cold block or nerve transection. In the use of fast axonal transport also affected any neuronal cells distal to the cold block, we examined expression of Po and p75 NGF receptor receptor, both of which are regulated as a function of Schwann cell:axon contact. Levels of p75 NGF receptor mRNA and protein were unaffected by the cold block. In contrast, levels of Po mRNA were decreased in the distal nerve in a fashion similar to that observed following axotomy. These data therefore suggest that neurons normally monitor the status of their axons and connections as a function of fast axonal transport, and b) that alterations in expression of Po and p75 NGF receptors result from different aspects of Schwann cell:axon communication, one of which involves fast axonal transport.

562.2
AMPHETAMINE INDUCED ROTATIONAL BEHAVIOR IN NON-LESIONED RATS: A ROLE FOR C-FOS EXPRESSION IN THE STRIATUM. B.J. Chiasson*, M.L. Hooper and H.A. Robertson, Dept. of Pharmacology, Dalhousie University, Halifax, N.S. Canada B3H 4H7.

Unstressed (i.p.) and their rotational behavior was monitored for two hours. In contrast, levels of Po mRNA were decreased in the distal nerve in a fashion similar to that observed following axotomy. These data therefore suggest that neurons normally monitor the status of their axons and connections as a function of fast axonal transport, and b) that alterations in expression of Po and p75 NGF receptors result from different aspects of Schwann cell:axon communication, one of which involves fast axonal transport.

562.3

We have previously demonstrated that two members of the α-tubulin multigene family that encode virtually identical proteins are differentially regulated in mammalian neurons; expression of Tα1 mRNA is specifically confined to brain processes undergoing growth, whereas expression of Tα2 mRNA is constitutive. Furthermore, expression of Tα1 mRNA is upregulated by NGF both in vivo, and in cultured sympathetic neurons, whereas Tα2 mRNA levels are not altered. In order to address the regulatory elements responsible for inducing Tα1 mRNA in response to NGF, we have isolated the promoter region from a rat genomic library. The 5′ promoter region thus isolated has been fused to lacZ and CAT to direct appropriate expression in vivo, and in peripheral ganglia. At all timepoints the transgene appears to be expressed in at least some populations of central and peripheral neurons.

562.4
AMPHETAMINE INDUCED ROTATIONAL BEHAVIOR IN NON-LESIONED RATS: A ROLE FOR C-FOS EXPRESSION IN THE STRIATUM. B.J. Chiasson*, M.L. Hooper and H.A. Robertson, Dept. of Pharmacology, Dalhousie University, Halifax, N.S. Canada B3H 4H7.

Ungarstien (1971a) reported that rats having had a unilateral lesion to the nigrostriatal pathway responded to a D-amphetamine (A) challenge by rotating toward the damaged side. More recently, it has been demonstrated that A can influence the expression of immediate-early genes (IEGs), such as c-fos, in normal animals (Graybiel, Morstall & Robertson, 1990). Here we ask whether the expression of c-fos might be important in modulating the functional output of the striatal system in normal non-lesioned animals following an A challenge. To test this possibility we used oligo-dioxynucleotide (ODN) technology to selectively inhibit mRNA translation of c-fos. Under steroidic guidance (S) and antisense (AS) ODN's were infused unilaterally into the striatum of normal rats. At varying times post-injection, 10 and 22 h, animals were sacrificed (p.t.) and their rotational behavior was monitored for two hours. Subsequently, animals were sacrificed and immunocytochemistry for Fos-like protein was performed on transverse sections.
562.5 DIFFERENTIAL C-FOS ACTIVATION BY PATTERNED STIMULATION H-Z Shen*, R.D. Fields, and P.G. Nelson. Lab of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

The pattern of electrical activation is important for such examples of neuronal plasticity as LTP or regulation of muscle contraction properties. To test whether immediate early gene expression is sensitive to the pattern of electrical stimulation, dissociated neurons of mouse dorsal root ganglion (DRG) were stimulated with electrical pulses organized in various patterns. The expression of c-fos mRNA by DRG neurons was monitored with a semi-quantitative PCR assay with optical density reading of ethidium bromide stained gel. A constant number of stimuli (180) were delivered in 3 different patterns for 30 min; 1) steady 0.1 Hz; 2) 6 stimuli at 10 Hz delivered every minute; 3) 12 stimuli at 10 Hz delivered every 2 minutes. The steady 0.1 Hz produced a small increase in c-fos expression over control, unstimulated cultures (p<0.02; 22.5±3.8 vs 15.8±2.8, mean O.D.±SD), while bursts of 6 pulses at 10 Hz produced a significantly larger increase (168%, p<0.001; 54.8±3.8). By contrast, the relatively infrequent 12 pulse bursts at 10 Hz produced no significant increase in c-fos expression (14.8±1.9). These results show that different patterns of stimulation can differentially regulate transcriptional events in neurons. Hence our data suggest that the cascade leading to c-fos activation could represent a mechanism by which the information in patterned electrical activity is decoded.

562.7 EXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS. A. Randolph1, D. Ye1 and E. Friedman. Univ. of Michigan Med. Center, Ann Arbor, MI 48109, 1Univ. of Texas at San Antonio, San Antonio, TX 78284.

Human retinal pigment epithelial (HRPE) cells provide a good in vitro model for studying the action of insulin-like growth factor I (IGF I), a polypeptide with growth-promoting and insulin-like activity. HRPE cells express both IGF I and the type I and II IGF receptors. In HRPE cells, IGF I action is modulated by a series of high affinity binding proteins, designated IGFBPs. IGFBPs regulate the tissue compartmentalization of IGF I and its binding to the type I IGF receptor. Glucose, insulin and IGF I alter hepatic IGFBP secretion. In this study we examined the regulation of IGFBP gene and protein expression in HRPE cells by glucose, IGF I, and serum. HRPE cells were grown in MEM in the presence of either 5 to 300 mM glucose, IGF I, IGF I plus the IGF I receptor antagonist (d-tub.) or serum. After collecting conditioned media, total RNA was isolated from each condition. Human cDNA clones were obtained from Dr. S. Shimomura, La Jolla, CA (IGFBP 2,3,5) and from Dr. D. Powell, Houston, TX (IGFBP 1,4). RNA was analyzed by Northern blotting and RNA protection. The IGFBPs present in conditioned medium were characterized by ligand blotting. Under all conditions, IGFBP 3 mRNA constituted the major transcript in HRPE cells. Ligand blotting demonstrated the presence of glycosylated IGFBP 3 forms at 43-49 kDa. There was no effect on basal IGFBP 3 gene expression by 1) physiologic concentrations of glucose; 2) IGF I; or 3) α I R3 added to cells to block potential effects of constitutive IGF I production by HRPE cells. In contrast, IGF I gene expression was up-regulated, in a dose dependent fashion, by calf serum. These findings suggest that IGF I alone cannot regulate the production of its binding protein(s) and implicate a role for other trophic factors in the IGF I-IGFBP axis. Supported by NIH grant NS01381 (EF) and R01CA52592 (DY).

562.9 INCREASED EXPRESSION OF RAT ADRENAL TYROSINE HYDROXYLASE GENE BY IMMUNOSTIMULATION STRESS. E.L. Sabban*, R. Kvetnansky2, A. Circulating catecholamines and the activity, as well as mRNA levels, of tyrosine hydroxylase (TH) were measured in rats in response to heat shock (2 hr IMO) in the rabbit brain with striking regional differences (for review see J. Neurosci Res., 27, 247-255, 1990). In situ hybridization studies using riboprobes which can distinguish between constitutive and inducible members of the hsp70 gene family revealed the prominent induction of a 2.7 kb hyperthermia-inducible mRNA species at 1 hr in glial-enriched areas of the rabbit brain with little induction in neuronal enriched areas such as the hippocampus. Further studies have revealed that hsp70 gene expression is not only regulated in a spatial manner but temporal differences in expression are also observed. Several neuronal populations including hippocampal and cortical neurons show delayed induction of the hyperthermia-inducible hsp70 mRNA species at 5 hrs post-heat shock with decreases by 10 and 24 hrs. This temporal study has also been extended to the protein level using Western blot and immunocytochemical studies which have shown important roles in cellular repair and/or protective mechanisms in the nervous system. Supported by grants from MRC Canada.

562.6 SINESAL AND TEMPORAL DIFFERENCES IN THE DISTRIBUTION OF mRNA ENCODING HEAT SHOCK PROTEIN 70 IN THE RABBIT BRAIN IN RESPONSE TO HYPERThERMIA. P. Manzerra and I.R. Brown*. Dept of Zoology, Univ of Toronto, Scarborough Campus, West Hill, Ontario M1C 1A4, Canada.

Our previous studies have shown that hyperthermia induces the expression of a heat shock protein (hsp70) in the rabbit brain with striking regional differences (for review see J. Neurosci Res., 27, 247-255, 1990). In situ hybridization studies using riboprobes which can distinguish between constitutive and inducible members of the hsp70 gene family revealed the prominent induction of a 2.7 kb hyperthermia-inducible mRNA species at 1 hr in glial-enriched areas of the rabbit brain with little induction in neuronal enriched areas such as the hippocampus. Further studies have revealed that hsp70 gene expression is not only regulated in a spatial manner but temporal differences in expression are also observed. Several neuronal populations including hippocampal and cortical neurons show delayed induction of the hyperthermia-inducible hsp70 mRNA species at 5 hrs post-heat shock with decreases by 10 and 24 hrs. This temporal study has also been extended to the protein level using Western blot and immunocytochemical studies which have shown important roles in cellular repair and/or protective mechanisms in the nervous system. Supported by grants from MRC Canada.


In a first attempt to better characterize the noradrenergic neurons of the locus coeruleus (LC) at the molecular level we screened a bovine LC cDNA library with a bovine LC minus cerebellum subtracted cDNA probe. Two of the cDNA's isolated show enrichment in LC compared to other regions tested on bovine brain regional northern blots. DNA sequence analysis and GenBank database searches indicate that the more LC-specific clone probably encodes the bovine homologue of ezrin, a phosphoprotein found in cytoskeleton of microvilli, and that the more ubiquitous clone encodes osteonectin, an extracellular Ca2+-binding glycoprotein. In situ hybridization studies in sections of rat brain at the level of the LC show that the mRNA's for these two genes are concentrated in LC noradrenergic neurons, but are also present in certain other cell bodies, including motor neurons of the trigeminal nerve. Ezrin- and osteonectin-like immunoreactivity are also enriched in LC noradrenergic neurons. It will be interesting to determine the functions subserved by ezrin and osteonectin in LC neurons.

562.10 SULFATED GLYCOPROTEIN-2 (SGP-2, CLUSTERIN) AND THE CENTRAL NERVOUS SYSTEM (CNS): DETECTION OF SGP-2 mRNA IN THE NEUROGLIA AND DISCRETE POPULATIONS OF NEURONS. J.-G. Chabot*, M. Danik, D. Hassan-Gonzalez and R. Ouimet. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Verdun and Maisonneuve-Rosemont Hospital Research Centre, Department of Medicine, Montreal University, Montreal, Quebec, Canada.

We recently reported on the expression of SGP-2 mRNA in human gliomas, epileptic foci and rat brain tissues (Danik et al., PNAS 88, 8577-81, 1991). We now extended these findings and report an extensive analysis of the cellular distribution of SGP-2/clusterin transcripts in the adult rat CNS. The overall pattern was one of widespread expression with several regions showing strong hybridization signals. Analysis at the cellular level revealed the differential labeling of distinct cell types. Among the non neuronal elements expressing SGP-2 mRNA, ependymal cells forming the walls of brain ventricles were strongly labeled. Although glial cells showed a diffuse labeling throughout the neuropil, scattered highly labeled cells were observed in the optic nerve, the corpus callosum and the hippocampal fissure. Specific mRNA labeling was also seen in neurons of the habenular complex, substantia nigra, cerebellum, several hypothalamic and brainstem nuclei, and the gray matter of the spinal cord. Strong labeling was especially concentrated in the motoneurones of the spinal cord and the ventral horn of the spinal cord. It is thus clear that SGP-2 gene expression is not restricted to glial cells but is seen in specific populations of neurons. This suggests that SGP-2 (also known as clusterin) may be a multifunctional protein which may be implicated in events such as the packaging, processing and transport of neurotransmitters and neuropeptides, as well as in the lipid metabolism. Supported by MRC Canada.
Identification of insertion mutants of vasopressin mRNA in the homozygous Brattleboro rat.

The +/di phenotype of these VP neurons in the di/di rat suggests PCR, followed by cloning of VP cDNAs in an expression vector. The aim of the present study was to elucidate the apparently normal VP gene products together with the mutant.

The di/di rat expresses a mutant VP precursor with an altered C-terminal extension. The intermediate neurofilament, glial fibrillary acidic protein (GFAP), is an astrocyte marker that increases in the brain, following injury, and astrocyte marker genes are regulated, in a neuronal cell line. Regulation by RA suggests a role for carcinoid in neuronal differentiation. The time course of response indicated that the first significant increase in calbindin mRNA is at 3 hrs with a plateau of calbindin mRNA induction at 48 hours after RA treatment.

Co-treatment of D2/B cells with RA and either cycloheximide or actinomycin D completely blocked the increase in calbindin mRNA, suggesting that the early response gene zif268 may be involved in induction of calbindin-D28 transcriptional regulation.

The amount of total RNA of tissue varied in the five different spinal cord sections. After normalising for the total μg of RNA, the alpha 1 mRNA levels were also found to be elevated. A value of 1.0 to 1.15 fold elevation was observed from Section 1 to Section 2. Section 2 was 2.6 times higher, Section 3 was 4.5 times higher, Section 4 was 2.6 times higher, and Section 5 was 1.7 times higher. Our results show that alpha 1 mRNA levels are highest in the mid-thoracic level (T5-T8).

The spinal cord of 5 anesthetized young adult rats were rapidly harvested and divided into five sections. Section 1 (C1-C8), Section 2 (T1-T4), Section 3 (T5-T8), Section 4 (T9-T13), and Section 5 (L1-L5). Total RNA from each section was isolated using the method of Chomczynski and Sacchi, also blotted onto membranes, and hybridized with 32P-labeled riboprobe transcribed from the cDNA clone which codes for the alpha 1 isoform of (Na,K)-ATPase. The membranes were subjected to autoradiography, the autoradiographs were scanned, linear regression lines were plotted, and the slopes of the lines were compared.

The intermediate neurofilament, glial fibrillary acidic protein (GFAP), is an astrocyte marker that increases in the brain, following injury, death, and astrogliosis. Spinal cord GFAP mRNA by glucocorticoids in vivo (O'Callaghan et al., Brain Res.494:159-161, 1989; Nichols et al., Mol. Brain Res.7:1-7, 1990; Laping et al., Mol. Brain Res.10:291-297, 1991). The activity of GFAP mRNA in section 1 was 1.7 times higher. Our results show that alpha 1 mRNA levels are highest in the mid-thoracic level (T5-T8).

Co-treatment of D2/B cells with RA and either cycloheximide or actinomycin D completely blocked the increase in calbindin mRNA, suggesting that the early response gene zif268 may be involved in induction of calbindin-D28 transcriptional regulation.
562.17
REGIONAL DISTRIBUTION OF ACETYLCHOLINE RECEPTOR ALPHA-SUBUNIT mRNA IN THE RAT SPINAL CORD. M. Dauzvardis*, S. Sayers, R. Shahid, C. Trausch, T. Khan. Rehabilitation R&D Center, Hines VA Hospital, Hines IL. 60141
A cDNA clone for the alpha-subunit of the acetylcholine receptor (AChR) has been previously identified by J. Boullier (J. Neurosci. 5: 2353, 1985). Variations in the distribution of this specific mRNA have been studied in hindlimb muscles of the rat following transection of the sciatic nerve. The present study demonstrates the presence and distribution of AChR alpha-subunit mRNA in the different levels of the spinal cord. The spinal cords of five anesthetized young adult rats were rapidly harvested and divided into five sections each: Section 1 (C1-C8), Section 2 (T1-T6), Section 3 (T7-T12), Section 4 (L1-L5), and Section 5 (L6-S2). Total RNA was prepared using the method of Chomczynski and Sacchi. The total RNA was slot-blotted onto Gene Screen Plus membranes and the membranes were subsequently hybridized with a 32P-labeled riboprobe transcribed from 5'UTR cDNA clones coding for the AChR alpha-subunit. The membranes were subjected to autoradiography, the autoradiograms were scanned, linear regression lines were plotted (μg total RNA vs. integration units obtained from scanning the autoradiograms), and then the slopes of the lines were compared. The amount of total mRNA of tissue varied in the five different spinal cord sections. After normalizing for the total μg of RNA, the AChR alpha-subunit mRNA levels were also found to vary. Assigning a value of 1 to the slope obtained from Section 1, Section 2 was 3.8 times higher, Section 3 was 9.8 times higher, Section 4 was 1.8 times higher, and Section 5 was 5.3 times higher (see diagram below). Our results show that AChR alpha-subunit mRNA levels are highest in the mid-thoracic level (T5-T8). In situ hybridization experiments are underway to further localize expression of AChR alpha-subunit mRNA in the rat spinal cord.

Supported by funds from Veterans Administration. Rehabilitation R&D Service.

562.19
REGULATION OF ETHANOL-RESPONSIVE GENES IN NEURAL CELLS. M.W. Spangaro, N. Wilke, G. Gayer, W. Chin, S. Barhite, and M.F. Miles*. Ernest Gallo Research Center and Clinic, UCSF, San Francisco, CA 94110
We are currently exploring the hypothesis that changes in neuronal gene expression may underlie the phenomena of tolerance to and dependence on ethanol seen in chronic alcoholics. Our lab has identified a number of genes which characterize the molecular mechanisms underlying ethanol-effects on protein trafficking in mammalian cells. In an attempt to understand the complexity of ethanol-responsive gene expression, we have investigated the transcriptional and posttranslational events that occur in response to ethanol. Initial in vitro experiments have confirmed the existence of a specific transcription factor which binds to these sequences. Finally, we have also performed dose-response and mixing studies comparing ethanol and other known inducers of Hsc70, Grp78 and Grp94. These studies may add substantially to the understanding of central nervous system adaptation to ethanol as seen in alcoholism.

562.20
Our lab has generated a monoclonal antibody (mAb 3G2) that recognizes a subcellular antigen, the nuclear matrix protein, in rat cell lines and primary cultures. In order to further investigate the mechanism of action of AF64A in an in vitro model, we used the LA-N-2 cholinergic human neuroblastoma cell line to study the effects of AF64A on cholerae transferase (ChAT) activity and on the steady state expression of the N-myc RNA. Following 1 hr exposure to 25, 50 and 100 μM doses of AF64A, ChAT activity was significantly reduced by approximately 15, 25 and 30 % (p < 0.05), respectively. The addition of 1 mM choline or hemicholinium-3 (HC-3) inhibited the decrease of ChAT activity observed at 25, 50 and 100 μM doses of AF64A. The protective effects may be explained by competition (choline) and inhibition (HC-3) of the HACHT system by which AF64A enter cholinergic cells. Neural cells, using an 51 nucleotide protection assay and doses of 50, 100 and 250 μM AF64A, we observed decreased steady state levels of N-myc mRNA at 3 and 6 hours after drug removal. At t = 3 hr, N-myc mRNA levels were significantly reduced by 40 and 75% (p < 0.05) at doses of 100 and 250 μM, respectively. At the lower doses (50 and 100 μM), the levels of N-myc mRNA began to recover at t = 6 hr to their initial steady state levels. We are currently examining the possibility that induced changes in ChAT activity may be due to AF64A's effect on genes critical for the maintenance of a cholinergic phenotype.

Supported in part by NIMH Grant #MH42572 (L.H) and #CA47929 (L.C.E.)

563.1
COMPARISON OF DIVERGENT CATION BINDING TO BRAIN AND ERYTHROCYTE SPECTRINS. J.A. Babitch*, C. J. Walls and F. Wahlgren, Chemistry Dept., Texas Christian University, Fort Worth, TX 76129.
Previously we examined calcium binding to brain spectrin [J. Biol. Chem. 267 (1992) 4333]. Here we report differences in divalent cation binding between brain and erythrocyte spectrins which appear to relate to differences in spectrin function in these two tissues. Flow dialysis and equilibrium dialysis of erythrocyte spectrin revealed two binding components: high affinity, calcium-specific sites with Ka Values of 1 x 10^10 M and a low affinity (millimolar) divalent cation component. The entropy increase upon binding suggests that calcium stabilizes the native conformation of the polypeptides. These data suggest that calcium binding to red blood cell spectrin has become specialized to stabilize the cytoskeletal network and the cell under the stressful conditions of blood circulation, whereas brain spectrin, having fewer high affinity sites, responds to fluctuating calcium levels altering its interactions with other proteins. Supported by NINH (NS-26518).

563.2
NEURAL DEPENDENCE AND INDEPENDENCE OF THE EXPRESSION OF A NMJ-ASSOCIATED ANTIGEN. Stephanie H. Astrow*, Young Jin Son and Wesley J. Thompson, Dept. of Zoology, Univ. of Texas, Austin, TX 78712.
Our lab has generated a monoclonal antibody (mAb 3G2) that recognizes a subcellular component of neuromuscular and myotendinous junctions in adult rats. On immunoblots, mAb 3G2 reacts with a relatively insoluble protein of 41 kD. To examine the neural regulation of this protein, we used the muscle denervation model, we used the LA-N-2 cholinergic human neuroblastoma cell line to study the effects of AF64A on cholerae transferase (ChAT) activity and on the steady state expression of the N-myc RNA. Following 1 hr exposure to 25, 50 and 100 μM doses of AF64A, ChAT activity was significantly reduced by approximately 15, 25 and 30 % (p < 0.05), respectively. The addition of 1 mM choline or hemicholinium-3 (HC-3) inhibited the decrease of ChAT activity observed at 25, 50 and 100 μM doses of AF64A. The protective effects may be explained by competition (choline) and inhibition (HC-3) of the HACHT system by which AF64A enter cholinergic cells. Neural cells, using an 51 nucleotide protection assay and doses of 50, 100 and 250 μM AF64A, we observed decreased steady state levels of N-myc mRNA at 3 and 6 hours after drug removal. At t = 3 hr, N-myc mRNA levels were significantly reduced by 40 and 75% (p < 0.05) at doses of 100 and 250 μM, respectively. At the lower doses (50 and 100 μM), the levels of N-myc mRNA began to recover at t = 6 hr to their initial steady state levels. We are currently examining the possibility that induced changes in ChAT activity may be due to AF64A's effect on genes critical for the maintenance of a cholinergic phenotype.

Supported in part by NIMH Grant #MH42572 (L.H) and #CA47929 (L.C.E.)

SYNAPIC STRUCTURE AND FUNCTION I
563.3
THE RECEPTOR-ASSOCIATED PROTEIN GEPHYRIN IS A COMPONENT OF MANY SYNAPSES IN THE RAT CENTRAL NERVOUS SYSTEM. J. König, P. Langosh, P. Prior, B. Schmitt, and H. Betz (Spon: European Neuroscience Association) Dept. of Neurochemistry, Max-Planck-Institute for Brain Research, Deutschordestr. 46, 6000 Frankfurt 71, Germany

Gephyrin was originally identified as peripheral membrane protein which is localized at the cytoplasmic face of glycine receptor subunits of the rat spinal cord. Rat and immunofluorescence studies using two different monoclonal antibodies against gephyrin demonstrate its widespread distribution at synapses. Tubulin overlay and copolymerization studies revealed a high-affinity interaction of gephyrin with microtubules (MTs). From cDNA clones, the primary structures of different gephyrin variants have been deduced; these are distinguished by the presence of different inserts (C1-C4) within the amino-terminal half of the protein. Co-polymerization experiments using in vitro translated deletion mutants of gephyrin suggest that the invariant carboxy-terminal region is implicated in the MT interaction. Our data suggest an important role of this polypeptide in postsynaptic receptor toplogy and architecture.

563.4
OCURRENCE AND ENRICHMENT OF PROTEIN TYROSINE PHOSPHATASE (PTPase) IN A POSTSYNAPTIC DENSITY (PSD) FRACTION ISOLATED FROM ADULT RAT BRAIN. X. Wang1, K. Wu2,3, X. Xue, Y. Huang3, T.W. Kim1,2, and B.B. Black1,2 (Graduate Program in Physiology and Neuroscience, University of New Jersey, New Brunswick, N.J. 08854, 1Dept. of Neuroscience and Cell Biology, UMDNJ/Robert Wood Johnson Medical School, Picayune, N. J. 08854 and 2Dept. of Neuroscience, NYU, New York, N.Y. 10016). Protein tyrosine phosphorylation plays an important role in the regulation of cell growth and differentiation in developing brain. In addition, recent studies show high levels of protein tyrosine kinases (PTKs) at synapses. Whether tyrosine phosphorylation may be involved in the modulation of synaptic transmission and plasticity is not yet clear. The phosphorylation of substrate proteins is achieved by a balance of the activities of PTKs which phosphorylate and PTPase which dephosphorylate the substrate proteins. So, remarkably, synaptic PTPase remains to be identified and characterized. Since PTKs were found to be enriched in the PSD (a functionally important, disc-shaped postsynaptic structure attached to inner surface of postsynaptic membrane), we examined the existence of PTPase in this structure with Western blot analysis. Using a highly specific antibody against a 37-kDa human recombinant T-cell-activated protein tyrosine phosphatase (TCOC1TPase), we revealed that the antibody specifically recognized a 60 kDa polypeptide in synaptosomal homogenate (SO), synaptosomal membrane (SM) and postsynaptic density (PSD) isolated from cerebellum (CBL), olfactory bulb (OB) and cortical (CTX) cortex of the rat brain. There was at least a 20-fold enrichment of the 60 kDa species in the PSD fraction over H and SM, suggesting that the PTPase plays a role in postsynaptic mechanisms. Moreover, the 60 kDa protein exhibited differential expression in CBL, OB and OB and OB. We conclude that the 60 kDa phosphatase protein is a PSD component that may play a regulatory role in synaptic function.

563.5
IMMUNOREACTIVITY OF β-AMYLIDO PROTEIN PRECURSOR (β-APP) SEQUENCES AT THE POSTSYNAPTIC DOMAIN OF HUMAN NEUROMUSCULAR JUNCTIONS (NMJs). R.J. Alvarez, V. Askanas, W.K. Engel*. USC Neuromuscular Center, Los Angeles, CA 90017-1969. The amyloidogenic fragment of β-APP, β-amylido protein (β-AP) in the brain has received attention for its putative role in the pathogenesis of Alzheimer’s disease (AD). In the muscle, β-APP is recently demonstrated pathologic accumulation of βAP in vacuolated muscle fibers of patients with inclusion-body myositis (Askanas et al., Lancet 339:550-561, 1992). We have now immunolocalized 3 sequences of βAPP, C-terminal, amino acids 676-695 (C-βAPP), N-terminal sequence 45-62 (N-βAPP), and tandem βAPP sequence, at the NMJs in 16 normal human muscle biopsies (total 200 NMJs), using 4 well-characterized antibodies. In all biopsies, all the 3 sequences identified by α-bungarotoxin (α-BT) binding had very strong immunoreactivity (IR) for all 3 APP sequences. N-βAPP-IR compared exactly to co-localized bound α-BT and dystrophin-IR. β-APP-IR and C-βAPP-IR extended slightly deeper into the muscle fiber end-plate region than bound α-BT. By immunogold-EM, C-βAPP-IR was localized in the muscle fiber postsynaptic domain a) in small tufts along the muscle-fiber side of the folds and b) in the form of clusters on small bits of membrane scattered throughout the end-plate region. We suggest APP may have an important role in normal junctional biology, and possibly in some diseases affecting NMJs. (We are grateful to GG Glenner, DJ Selkoe, B Frangione and D Levartovsky for generous gifts of antibodies.)

563.6
A RAT POSTSYNAPTIC DENSITY PROTEIN, PSD-95, IS A HOMOLOGUE OF THE DROSOPHILA DISCS-LARGE PROTEIN AND CONTAINS A GUANYLATE KINASE DOMAIN. K.O. Cho, C.A. Hunt*, K.-O. Cho, and M.B. Kennedy*. Division of Biology, California Institute of Technology, Pasadena, CA 91125. To understand the structure and function of the postsynaptic density (PSD), we set out to study biochemical and molecular properties of several proteins in the PSD fraction from rat brain. We isolated cDNA clones encoding a protein of apparent Mr 95 kDa that is tightly associated with the PSD by screening a rat brain cDNA library with DNA probes designed from tryptic peptide sequences of the protein. The cDNAs encode a protein of 724 residues with a MW of 80,465. The deduced PSD-95 protein sequence has high similarity to the synthesis of GFP in Drosophila tumor suppressor protein, lethal (l). This sequence is 60 kDa, was found in the postsynaptic density (PSD). We conclude that the PSD-95 protein is a synapse-specific guanylate kinase phosphate synthase, binding against bacterially expressed PSD-95 show that it is enriched in PSD fractions. The same antibody stains dendrites of neurons in the cortex and hippocampus of rat brain. PSD-95 mRNA was only detected in brain, suggesting that PSD-95 is a brain-specific guanylate kinase. The phenotype of Drosophila disc mutants suggests that GSP36 synthesis of these proteins may be regulated by external signals and that the locally regulated GDP-GTP ratio in turn regulates G-protein such as ras.

563.7
DISTRIBUTION OF THE POSTSYNAPTIC DENSITY PROTEIN, PSD-95, IN RAT BRAIN. J.A. Garner*, K.-O. Cho, and M.B. Kennedy. Division of Biology, California Institute of Technology, Pasadena, CA 91125. This laboratory has recently cloned a cDNA encoding a prominent protein in rat postsynaptic density fractions, of apparent molecular weight 95kD, termed PSD-95. It is highly similar to the Drosophila lethal(discs-large-1) (dlg) tumor suppressor protein, which is associated with septate junctions in developing flies. Conflont immunofluorescence and peroxidase immunohistochemistry with an affinity-purified rabbit polyclonal antiserum to PSD-95 have shown that the protein is present in neurons in many regions of rat brain. Thionin-counterstaining of 15 micrometer sections suggests that all neurons contain PSD-95. In hippocampus, dentate granule cells and pyramidal neurons in CA1 and CA3 are stained, as well as mossy fibers in CA3, while most interneurons appear unstained. In neocortex, most neuronal layers are strongly stained with the PSD-95 antiserum, except layer II-III and V-VI; staining is particularly intense in layer V pyramidal neurons. Fewer immunoreactive neurons are present in layer IV. In both hippocampus and neocortex, PSD-95 immunoreactivity is absent from cell nuclei and is more intense in dendrites than in somata. Staining is not homogeneous in dendrites, but is concentrated in small discrete spots, consistent with the presence of binding sites for the 95 kDa protein. PSD-95 is also highly concentrated in postsynaptic densities. In cerebellum, PSD-95 does not stain Purkinje cell somata, but is present in somata and dendrites of stellate cells in the molecular layer. The number of neurons stained decreases from the Purkinje cell layer into the molecular layer. Considering each Purkinje cell and concentrated at its basal end, suggesting that basket cell axons synapsing onto Purkinje cells contain high levels of PSD-95.

563.8
PRESYNAPTIC CONTRIBUTION OF A 130 kDA PROTEIN TO POSTSYNAPTIC DENSITY PREPARATIONS. J.A. Garner*, USC School of Medicine, Los Angeles, CA 90033. The morphological correlate of vertebrate CNS synapses, synaptosomes, can be enriched by subjecting homogenized brain tissue to sucrose density gradient fractionation. The isolated synaptosomes contain most pre- and postsynaptic features of synapses, and release neurotransmitter in a calcium-dependent manner. Synaptosomes isolated from cerebral cortex were pre-warmed before the fractionation scheme developed for enriching the postsynaptic density. A single labeled (presynaptic) protein at ~130 kDa, was found in the fraction enriched for postsynaptic density structures. Whether this protein is actually incorporated within the postsynaptic structure, or is a presynaptic remnant of the synaptic cleft peripherally associated with the postsynaptic density is not yet clear. The extraction of transported proteins in the presynaptic axon reveals approximately equal distribution of this protein in the aqueous and detergent phases. Once the protein reaches the terminal region, it is found almost exclusively in the detergent or hydrophobic phase.
563.9
EXCLUSIVELY NEURONAL LOCALIZATION OF THE SYNAPESE PROTEIN SNAP IN DROSOPHILA MELANOGASTER.
C. Riesen, Y. A. Pieribone, D. Nüssel, A. Lambrettson, L. Brodin, and I. Gustafson. Dept. of Medical Genetics, Box 560, Uppsala University, S-751 23 Uppsala, Sweden. Nobel Inst. f. Neuroscience, Inst. of Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030

Synaptobrevin, an integral membrane component of synaptic vesicles, is well conserved from invertebrate through vertebrate evolution and has been suggested to play a role in synaptic vesicle docking and fusion. To investigate the synaptobrevin function further, we have examined synaptobrevin's developmental expression in Drosophila and have initiated a genetic analysis of the gene. Low stringency screens to identify additional synaptobrevin cDNAs derived from Drosophila embryos failed to isolate isolomers differing from the adult message, suggesting the presence of a single synaptobrevin gene. While means to hybridization of Drosophila embryos indicate that synaptobrevin transcription begins in the central nervous system during stage 13, and in the peripheral nervous system during stage 15. Synaptobrevin message appears in all neurons of the CNS, and in most, if not all, CNS neurons. Antibodies prepared to bacterially produced recombinant Drosophila synaptobrevin and to an amino-terminal synaptobrevin peptide, recognize a 70 kD protein on western blots of crude Drosophila synaptic vesicles. Antibody staining of embryos indicates that protein expression begins in the CNS and PNS during stages 14 and 16, respectively. Antibodies to synaptobrevin strongly label the two longitudinal tracts in the central nervous system and brain, as well as many other areas of the CNS. In addition, the antibody recognizes many peripheral neurons, with intense staining localized to the neuromuscular junction. We have localized the synaptobrevin gene to the second chromosome at 23B-C, and have identified a deficiency (23AI-21C) to which synaptobrevin maps. Embryos or first instar larvae with this deficiency are homozygous lethal and are the subject of further work to define the consequences of loss of synaptobrevin expression on neurotransmission. These data indicate that synaptobrevin expression in Drosophila is widespread in both the peripheral and central nervous systems and correlates to a period of development when synaptic activity begins to occur.

563.11
SYNAPTIC VESICLE PROTEINS IN DROSOPHILA. A. DiAntonio, R.W. Burgess, and T. L. Schwartz. Dept. of Molecular and Cell. Neuroscience, Inst. of Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030

SNAP (synaptotagmin-associated protein) is a 25 kDa protein which is expressed exclusively in neurons in mammals. It is located in synaptic nerve terminals and it is associated with the inner side of synaptic vesicle membranes. These proteins have been termed synaptic vesicle proteins in Drosophila. A. DiAntonio.

It is assumed that SNAP proteins are not ubiquitous, as they are expressed in specific neuronal subpopulations. We have previously shown that SNAP is an extremely well-conserved protein which implies important functions. The chicken and mouse SNAP proteins are identical throughout the 206 amino acids (S. Cattel et al., PNAS 86, 785-789, 1991), and SNAP of goldfish, ray (Torpedo marmorata), and lamprey, and Drosophila melanogaster show extensive similarity to the mouse protein (Abstract 154.14, NM 1990). The Drosophila SNAP gene has a complex structure with eight exons spanning more than 65 kbp (Abstract 458.6, NM 1991).

We have now started to explore the anatomical and temporal aspects of SNAP expression in Drosophila. Northern blot analysis reveals a single mRNA of approximately 2.5 kbp in both larval and adult flies. In situ hybridization to Drosophila tissue sections gives specific labeling to neuronal cell bodies in the brain and in the ventral ganglion. No specific binding to the intestine or the muscles was observed.

Thus, SNAP is highly conserved protein exclusively expressed by neurons in Drosophila. The large size of the gene may indicate an important role in the development of the nervous system.

563.13
SYNAPTIC VESICLES IN GLUTAMATE, GABA AND GLYCINE-IMMUNOREACTIVE SYNAPES IN THE LAMPREY SPINAL CORD HAVE DISTINCT MORPHOLOGICAL CHARACTERISTICS. D. O'Sullivan*, M. Brodin, O. O'Connor* and J. Storm-Mathisen* Nobel Institute for Neurophysiology (1), Department of Anatomy (2) Karolinska Institute, Stockholm, Sweden and Anatomical Institute (3), University of Oslo, Oslo, Norway.

The lampey spinal cord is particularly useful to study the regional distribution of amino acids and peptides, as axon collaterals and compartments in most axons can be anatomically separated. The ultrastructural distribution of glutamate, aspartate, GABA and glycine immunoreactivity was analysed in spinal cord fixed in 2% glutaraldehyde, 0.1 M phosphate buffer using the immunogold postembedding technique. An accumulation of immunoreactivity over clusters of synaptic vesicles was revealed for GABA, glutamate and glycine in the respective immunoreactive axons, but not for aspartate. Hence, only the former three amino acids can be assumed to act as neurotransmitters. The labelled synaptic junctions displayed distinct morphological characteristics. Axons with GABA-ir vesicle clusters contained spherical to pleomorphic vesicles, numerous glycogen granules and some dense core vesicles, while glycine-ir vesicles contained flattened vesiicles. Axons with glutamate-ir vesicle clusters contained spherical vesicles. Glutamate-ir axons formed asymmetrical synapses, which often contained gap junctions. Glycine-ir synapses were symmetrical, while GABA-ir terminals established both symmetrical and asymmetrical synaptic junctions.

The results emphasize that axons utilizing different amino acids as neurotransmitters exhibit unique inclusions in glutaraldehyde fixed tissue. The distinct shape of vesicles in these axons will significantly simplify the structural analysis of neuronal circuits in the lampey spinal cord.
SEGREATION OF CHEMICALLY-DEFINED VARIocities UPON PARTICULAR Dendrites OF A NEUron: A NOvEL MORPHOlOGICAL BASIS FOR NEURonal MODULATION? D. R. Onstott* and A. J. Beitz, Univ. of Minnesota, St. Paul, MN 55108

The segregation of chemically-defined synaptic inputs to neurons based upon their proximity to the cell soma has been examined in several studies. However, we are unaware of any reports documenting the segregation of such inputs on particular dendrites of a cell. Here we report selective substance P (SP) innervation of portions of the dendritic tree of a neuron in the periaqueductal gray (PAG). Retrogradely labeled PAG neurons were injected intrathecally with Lucifer yellow (LY), and SP immunoreactivity was detected in the same sections using fluorescence immunocytochemistry. Separate series of optical sections of injected cells and of SP-immunoreactive (SP-IR) varicosities were produced using confocal laser microscopy. Individual, coplanar images from each series were merged and the site of apposition of each SP-IR varicosity was marked on LY-filled neuronal structures. 1.5 μm images were then merged to produce a projection of the entire neuron. Some cells and the corresponding series of SP images were also reconstructed into stereo pairs, merged, and appositions identified independently on the three dimensional images. Among the 10 cells thus far studied in detail, three have exhibited SP-IR appositions that appear to be preferentially associated with one or more dendrites. Some dendrites of each cell exhibited a high innervation density (x=5.7 appositions/100 μm), while other dendrites exhibited no SP innervation or low innervation density (x=1.4 appositions/100 μm). These results suggest that chemically-defined inputs to neurons may be spatially segregated by selectively contacting certain dendrites of an individual cell. This pattern may be of functional significance in providing a morphological basis for differential modulation of cellular activity. Alternatively, it may represent the most efficient configuration for providing multiple contacts with adjacent cells. Supported by DA06687, DB06642 and DC10806.
THURSDAY PM

SYNAPTIC STRUCTURE AND FUNCTION II

564.5

1339

564.6

SYNAPTIC E N H A N C E M E N T BY LOCAL PR ESY N A PTIC
STIMULATION AFFECTS THE SHORT-TERM PLASTICITY OF
INDIVIDUAL RETICULOSPINAL EPSPs DIFFERENTLY
L. Brodin,* O. Shupliakov and V. Pieribone, The Nobel Institute for
Neurophysiology, Karolinska Institutet, S-104 01 Stockholm, Sweden
The short-term plasticity of mixed electrochemical EPSPs evoked in lamprey
spinal neurons by single giant reticulospinal nerve cells was studied using
paired-pulse stimulation (interval 65 ms). During stimulation of cell bodies the
amplitude of the second chemical EPSP (kainate/AMPA-mediated, AP-5
present) was always larger than the first EPSP, i.e. facilitation predominated
over depression, while the electrical component was unaltered. The degree of
facilitation of the chemical EPSP varied considerably in different spinal
neurons, from a slight increase to more than a two-fold increase, even among
EPSPs evoked by the same presynaptic neuron. When axons were impaled
and stimulated close to the synaptic area (about 100-500 pm), the postsynaptic
response was found to depend strongly on the stimulation parameters. The
amplitude of the chemical EPSP increased if the depolarizing current pulse
used to trigger action potentials was increased, or if the distance between the
stimulating electrode and the synapse was decreased. This synaptic
enhancement could alter the EPSP plasticity, such that less paired-pulse
facilitation occurred. In many cases the second EPSP even had a lower
amplitude than the first, i.e. depression predominated over facilitation, while
both electrical components had the same amplitude. However, an
enhancement of the EPSP did not always alter the EPSP plasticity. In some
cases a pronounced facilitation remained, albeit the amplitude of the first
EPSP was markedly increased. These data imply that individual reticulospinal
synapses have different plasticity properties which, at least in part, depend on
mechanisms linked to the presynaptic element.

SIZE AND NUMBER OF ACTIVE ZONES IN M AUTHNER CELL
INHIBITORY AFFERENTS INCREASE FROM SOMA TO
M orphological characteristics of inhibitory active zones were analysed at
the level of M authner cell’s (M-cell) axon-cap, soma, and the medial and
distal parts of the lateral dendrite (150-200 and 350-450 pm away from
axon-cap, respectively). For this purpose, presynaptic grids were stained
with ethanolic phosphotungstic acid (EPTA). Both glycine and GABA
were visualized by post-embedding immunogold labeling on semi-thin (0.5
pm) sections. In the axon-cap and soma, the afferent boutons have small
sized glycinergic presynaptic grids with respective areas of 0.066 ± 0.02
μm2 , (mean ± S.D ., n = 30), and 0.075 ± 0.03 μm2, (n = 46). 96% of these
terminals in the axon-cap and 82% of these on the soma have only one
active zone; the rest are mainly boutons with two grids. On the dendrite,
both glycinergic and gabaergic dendritic afferents have larger release sites.
Specifically, the surface of each active zone is increased to 0.135 ± 0.08
μm2, (n = 113; GABA), 0.141 ± 0.08 μm2, (n ≈ 148; glycine) on the middle
portion and 0.139 ± 0.08 μm2, (n = 125; GABA), 0.147 ±0.1 μm 2, (n = 115;
glycine), on the distal dendrite. These values are similar for both gabaergic
and glycinergic boutons, and are significantly different from somatic ones
(Student t tests). Furthemore, the proportion of profiles displaying two
(31% for GABA; 30% for glycine) and three to four (4% for GABA; 7% for
glycine) active zones, is greater on the dendrite than on the soma. These
results suggest that the probability of transm itter release is higher at
dendritic inhibitory afferents than at somatic ones. Such could also be the
case for the size of quanta when occurring at more widespread
postsynaptic receptor aggregates.

564.7

564.8

BACKFIRING AT MIXED SYNAPSES ON THE MAUTHNER (M) CELL.

Alberto Pereda* and Donald S. Faber. Dept. of Physiology, SUNY-Buffalo,
Buffalo, N Y 14214.
Single eighth nerve afferents terminate on the M-cell’s lateral dendrite
as large myelinated club endings which have both gap junctions and chemical
synapses with the cell. Low threshold extracellular stimulation of the posterior
branch of the eighth nerve produces a mixed excitatory post-synaptic
potential (Vlllth EPSP) consisting of a fast electrotonic potential, followed by a
chemical glutamatergic component. Intracellular recordings of these afferents
were obtained, alone or while simultaneously recording from the M-cell.
Membrane potential ranged from -65 to -73 mV. Coupling potentials can be
recorded from these afferents either when the M-cell is activated antidromically
by stimulation of its axon in the spinal cord or when an Vlllth stimulus is
subthreshold for the impaled axon. In the latter case, an attenuated composite
Vlllth nerve evoked EPSP is recorded in the afferent. Backfiring of these
afferent fibers was obtained in a number of situations: i), following stimulation
of the Vlllth nerve at a strength subthreshold for the recorded axon, ii), pairing
a weaker Vlllth nerve stimulus with antidromic activation of the M-cell, and iii),
pairing the antidromic stimulus with a presynaptic depolarizing current. We also
found that the coupling potentials recorded from a fiber are voltage
dependent, increasing with fiber depolarization and decreasing with
hyperpolarization. Current/voltage relationships obtained from the fibers
suggest that both membrane and junctional properties may be involved.
Backfiring associated with physiological activation of eighth nerve afferents
could serve as a positive feedback, recruiting additional fibers and enhancing
the EPSP in the M-cell. It could also impart a significant non-linearity to the
input-output relationship of the population response.

(Supported by a Buswell Fellowship to AP and by NIH grant NS15335).

564.9
M IN IA T U R E E X C IT A T O R Y S Y N A P T IC C U R R E N T S (m E P S C s )
IN M O T O N E U R O N S (M N ) O F O R G A N O T Y P IC R A T S P IN A L
C O R D D O R S A L R O O T G A N G L IO N C O C U L T U R E . D .Ulrich* and
H .-R . L ü s c h e r, D e p t. of P h ysio lo g y, U n ive rs ity of B e rn , 3 0 1 2
B ern , S w itze rla n d
In o rd e r to study th e q u a n ta l e v e n ts at cen tral syn a p s e s
m E P S C s w e re reco rd e d fro m v o lta g e c la m p e d M N s in w h o le cell
co n figuratio n a fte r bath ap p licatio n of 3 μ M T etro d o to xin , 3 μ M
S trych n in e, 1 0 p M B icucullin e a n d 1 0 0 μ M D -2 -A m in o -5 p h o s p h o n o p e n ta n o ic acid (D -A P V ) in 2 m M C a ++. C e lls w e re
id entified m orp h o lo g ically a n d im m u n o h is to c h e m ic a lly with a
m onoc lon al a n tib o d y a g a in s t C h o lin e a c e ty l-tra n s fe ra s e . T h e
m E P S C s w e re reversib le blo cked w ith 1 0 μ M of th e co m p e titive
n o n -N M D A rec e p to r an ta g o n is t 6 -C y a n o -7 -n itro q u in o x a lin e -2 ,3 d io n e (C N Q X ). A d etectio n algorithm s e le c te d individu al currents
fro m con tin u o u s reco rdings s a m p le d a t 10 k H z . M o st m E P S C s
cou ld b e fitted b y th e su m o f tw o e x p o n e n tia ls of w hich am p litu d e,
halfw idth a n d ris etim e w e re c a lc u la te d . A m p litu d e histogram s
w e re s k e w e d to w a rd s la rg e r e v e n ts . Individu al p e a k s w e re not
u n a m b ig u o u sly reso lv a b le . T h e m e a n of th e m o d e s w a s -1 9 pA
(S D = 7 .7 pA , n = 8 ) w ith a m a xim al ra n g e of -4 p A to - 1 6 0 pA.
A m p litu d e distributions c o m p ile d fro m c u rren ts w ith identical
risetim e o r from th e w h o le p o p ula tion w e re sim ilar. W e co n clu d e
th at th e b ro ad ra n g e of m E P S C s a m p litu d e s c a n n o t b e e x p la in e d
by electro to n ic a tte n u a tio n o f cu rren ts fro m d iffe re n t den dritic
location s. ( S N F 3 1 -2 7 5 5 3 .8 9 )

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992

TEMPORAL INTERACTION OF SYNAPTIC INPUTS IN VAGAL
MOTONEURONS.
R. N itzan, I. Segev & Y. Yarom*. Dept. of
Neurobiology, Hebrew University, Jerusalem, Israel.
Temporal synaptic interactions in motoneurons from the dorsal motor vagal
nucleus (DMVN) of guinea pigs were studied in brain stem slices. The submerged
slice technique and conventional intracellular recordings were used in this study.
Synaptic responses were elicited by stimulating non-specific presynaptic axons at
the nucleus surroundings (peri-vagal stimulus, Yarom et al. Neurosciense 16:4,719737, 1985). The temporal interactions were studied by analyzing the responses to
trains of stimuli at various inter-train intervals that where delivered at different
frequencies. At inter-train intervals longer than 10 msec and a frequency of 0.2 Hz,
the peaks of all synaptic potentials in a train tend to reach the same level.
Summation of synaptic potentials occurs only at shorter inter-train interval. The
decay phase of the response to a train of stimuli (in most cells) is governed by two
time constants (20msec, 100msec). Both are slower then the passive membrane
time constant (τ0= 10msec). This finding indicates that the train of stimuli activates
a long lasting conductance change. Trains of stimuli, delivered at frequencies of
0.25 Hz or higher, induced synaptic potentiation that lasts for 30 sec. Ten trains of
5 stimuli each, elicited at a frequency of 0.25Hz, were sufficient to double the
amplitude of the synaptic potential but did not change the pattern of the temporal
summation. A detailed model of DMVN motoneurons (Nitzan et al. J.
Neurophysiol. 63: 333-346, 1990) was used to study temporal interaction in these
cells, assuming passive dendrites. Using this model we estimated the non-linearity
of synaptic interactions in these motoneurons by comparing the temporal
summation as preformed by the actual cell and that expected in a passive model. The
results suggest that a bursting input to DMVN motoneurons is more effective than
a single input only if the bursting occurs at a frequency around 0.2 Hz, where the
response is potentiated.

564.10

MODULATION OF SPONTANEOUS EPSPS IN RAT MOSSY CELLS.
B.W. Strowbridge* and P.A. Schwartzkroin. Depts. of Physiology & Biophysics,
and Neurological Surgery, Univ. of Washington, Seattle, WA 98195.
We have carried out a series of experiments to characterize spontaneous
postsynaptic potentials (PSPs) in rat mossy cells and their modulation by
intracellular depolarization. Spontaneous PSPs appear to be almost exclusively
excitatory since they are blocked by the non-NMDA receptor antagonist, CNQX
(10-50 uM) and alterations of the membrane potential consistently fail to reveal
hyperpolarizing synaptic potentials. Previous studies suggested that at least
some spontaneous EPSPs may be due to active granule cell terminals,
discharging independently of the usually hyperpolarized soma. In four mossy
cells, we observed that bath application of TTX (1 uM) dramatically reduced
(but did not abolish completely) the frequency of spontaneous EPSPs,
suggesting that granule cell axons and/or terminals can generate sodium spikes
spontaneously. However, unlike in hippocampal pyramidal cells, TTX-resistant
EPSPs in mossy cells were quite large (3-4 mV), of a magnitude similar to that
observed before treatment (up to 10-15 mV).
We also have obtained evidence that spontaneous EPSPs can be modulated by
depolarization of the postsynaptic neuron. We previously demonstrated that
treatments which result in depolarization of mossy cells, including direct current
injection into a single neuron, can potentiate both the amplitude and frequency
of spontaneous EPSPs for prolonged periods. We now report that intracellular
injection of the calcium chelator, BAPTA, significantly deceases the basal level
of spontaneous EPSPs, and that strong (1-2 nA) depolarizing current pulses
(similar to those which potentiated spontaneous EPSPs) evoke calcium spikes.
These data support a role for postsynaptic calcium levels in the modulation of
spontaneous EPSPs in mossy cells.
Supported by NIH grants NS20482 and NS07097.


SYNAPTIC STRUCTURE AND FUNCTION II
THURSDAY PM

564.11 LOCAL CIRCUIT, SINGLE AXON EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPs) IN DEEP LAYER NEOCORTICAL PYRAMIDAL NEURONES.
A.M. Thomsen*, D.C. West and J. Deuchars. Dept. Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

Previous studies of single axon pyramidal connections in neocortex concentrated on layers II/III and IV. EPSPs exhibited a relatively rapid time course, despite an unconventional voltage relation and frequency-dependent potentiation with post synaptic depolarization beyond -70 mV and limited fluctuations in amplitude. The depression in average amplitude with increases in presynaptic firing rate or with paired pulse depression, were consistent with a decrease in presynaptic release probability. The present study aimed to determine whether the properties of connections between deeper pyramidal neurones were similar. Simultaneous intracellular recordings were obtained from pairs of neurones in rat neocortical slices. Pyramidal neurones were identified electrophysiologically and subsequently, morphologically on histological examination. All properties typical of superficial local circuit EPSPs were apparent. The only clear difference, at this stage, was the higher probability with which EPSPs of >1 mV average amplitude were encountered in deeper layers. This was approximately 1 in 50, compared with 1 in 100. In addition, the largest events could be >7 mV in amplitude.


Lizard (Anolis carolinensis) intercostal muscle miniature endplate currents (MEPCs) were recorded under 3 conditions: unblocked, acetylcholineesterase (AChE) inhibition, and AChE inhibition + 20% nicotinic acetylcholine receptor (nAChR) binding site blockade. Mean amplitude (+SD) 20-80% rise time (t_r), and e-fold fall time (t_f) for each condition were the same for 35 units. Half width = 50% clear failures. Mean variance was < 5% by Mann Carlo MEP computer simulations. Fixed model features included: geometry of 1st clef and junctional folds. mACHr site density, identical ACh binding sites, and single ligand open state; rate constants for AChE association and hydrolysis. We performed simulations iteratively to determine parameter sets corresponding to different assumed values of g_a, g_m, g_mACh, but not 50% blocked receptors for channel closing and opening, respectively. Each parameter set included: quantal size (N); ACh diffusion coefficient; rate constants for AChR association (k+); and dissociation (k-); mACHr site density (σ). For each parameter set we also quantified molecular binding, unbinding, opening and closing events. For 0.8 < g_a < 1, parameter set values all fell within suggested literature ranges, but nonetheless varied between sets from <20% (σ) to >20-fold (k-). Based on preliminary MEPA shape analysis, the optimal value for g_a was 0.95. With AChE active g_a exceeded mean burst duration by less than 30% yet single AChs bound AChR multiple times (e.g. 2.4 times at g_a = 0.8, and ~5.0 times at g_a = 0.95). Clearly, channel opening probability (β) and k- decreased dramatically as g_a was increased, even though MEPC efficiency (A/CN) increased. Furthermore, ACh binding frequency was sensitive to σ variation in the range in which ACh, t_r, and g_m were little affected. This suggests that trophic ACh/nAChR interactions could be regulated through σ through compromising efficiency of electrical signal transduction.

Supported by NIH grants NS09315 (M.M.S.) and F32NS09126-01 (J.R.S.). Simulations were conducted at the Cornell National Supercomputer Facility.

564.13 ISOLATED SNAKE NEUROMUSCULAR BOUTONS RELEASE 2-3 QUANTA WHEN ACTIVATED. R.S. Wilkinson* and S.D. Lunn. Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

Despite their importance as the anatomical substrate of quantal release, little is known about the function of individual synaptically active boutons, in part because axon terminals typically comprise many synchronously active bottons. To study quantal release from a single bouton, we used a technique which permits dissociation of living boutons from both their motor nerve terminal and their postsynaptic (endplate) site. The individual boutons may be studied either in isolation, which provides access to the presynaptic membrane, or after attachment to vacant endplate sites, for quantal analysis of reconstructed one-bouton NMJs.

Boutons became near-spherical when micromanipulated from their endplate site with a glass suction pipette. A second pipette (loose patch configuration) was used for stimulation and recording. Boutons remained healthy for >1 hr, as evidenced by uptake of the supravital probes 4-Di-2-ASP (mitochondrial) and sulforhodamine 101 (activity-dependent), and by spontaneous quantal release (MEPs recorded from reconstructed NMJs). When stimulated, boutons exhibited 4-aminoypyridine-sensitive terminal currents similar to those recorded from boutons of intact NMJs. Transmitter release (0.2 Hz stimulus rate) underwent quantal fluctuations about a mean of 2-3 quanta, with occasional failures. Maximum observed quantal content was 7. Serial EM of similar boutons (from intact NMJs) revealed 6-7 active zones, suggesting that the probability of release was 0-3.3-4. Supported by NIH grant NS24752 and the MDA.

564.15 TRANSMISSION-DEPENDENT DEPRESSION COEXISTS WITH FACILITATION AT HIPPOCAMPAL EXCITATORY SYNAPSES. Johan F. Storm*, Inst. of Neurophysiology, University of Oslo, Norway.

Hippocampal synapses show facilitation, which appears to be presynaptic since it is independent of prior synaptic transmission (Neurosci. Abstr. 17, p.1486). Here we report that these synapses also show depression, which is dependent on presynaptic transmission, and usually masked by the facilitation. CA1 pyramidal cells in slices from young rats were voltage-clamped (whole-cell) at -70 mV, with 2 mM Ca and 10 μM bicuculline in the bath. Pairs of minimal stimuli (ST1 and 2, interval 40-60 ms) from a glass pipette in str. radiatum, evoked synaptic currents (EPSC1 and 2) of variable amplitude, duration and e-fold fall time (tr), and e-fold fall time (ε) from reconstructed NMJs. When recording from many synapses obscures the underlying quantal nature of transmission. Accordingly, we attempted to minimize possible synaptic heterogeneity by performing quantal analyses on small numbers of active synapses.

Dual whole-cell recordings were made from pairs of CA1 neurones. The bath solution contained 5 mM calcium to block transmission at the majority of synapses, while a puff-suction arrangement was used to locally apply bath solution containing 5 mM calcium to short lengths of dendrites. After many sweeps were recorded, a separate puff was used to apply hypotonic bath solution to the same region, eliciting miniature EPSCs. Finally, Synaptin I antibody was used to count the number of synapses within the activated region.

The synapse count ranged from 5 to 20 in 13 experiments. Peaks were often visible in the amplitude histogram as discrete peaks in histograms of miniature EPSCs. The only deviation, at this stage, was the higher probability of release, which arises from the presynaptic bouton.

Supported by NIH grant NS09315 (M.M.S.) and F32NS09126-01 (J.R.S.). Simulations were conducted at the Cornell National Supercomputer Facility.

564.16 QUANTAL ANALYSIS OF EPSCS RECORDED FROM SMALL NUMBERS OF SYNAPSES IN RAT HIPPOCAMPAL CULTURES. J.M. Bekkers* and C. F. Stevens. Division of Neuroscience, JCSMR, Institute, Howard Hughes Medical Institute, La Jolla, CA 92037.

Current evidence supports the notion of quantal synaptic transmission in central neurons, as revealed by the presence of discrete peaks in histograms of synaptic amplitudes. However, we were unable to resolve such peaks when recording EPSCs from cultures of profusely-connected hippocampal neurons. Mean amplitude was little affected. This suggests that trophic ACh/nAChR interactions could be regulated through σ through compromising efficiency of electrical signal transduction.

Supported by NIH grants NS09315 (M.M.S.) and F32NS09126-01 (J.R.S.). Simulations were conducted at the Cornell National Supercomputer Facility.

564.17 HOW MANY VESICLES ARE RELEASED AT A CENTRAL SYNAPSE? N. Hessler*, R. Malinows, Neuroscience Program and Dept. of Physiology and Biophysics, University of Iowa.

We are addressing this question by looking at the AMPA and NMDA components of elicited excitatory postsynaptic currents in hippocampal slices under various release conditions. Under normal release conditions we measure the NMDA/AMPA ratio (synaptic amplitude at 40 ms/5 ms). We apply an APV concentration to produce a 50% decrease in this ratio. After washout of APV, we add an agent to the bath that increases the release of transmitter (Ca²⁺-activated release). We then measure the NMDA/AMPA ratio under increased release conditions. If multiple vesicles are released at a synapse, and NMDA receptors are saturated, then one would expect a decrease in the NMDA/AMPA ratio with increased transmitter release. We then apply APV at a concentration that produced a 50% reduction in the NMDA/AMPA ratio under normal release conditions. If NMDA receptors are saturated we expect less than a 30% reduction in the NMDA/AMPA ratio during increased release conditions. On the other hand, if a single vesicle is released per synapse, then increased release probability will merely recruit more synapses, and the NMDA/AMPA ratio should not change. APV should have the same relative effect in normal as high release conditions.
564.1


Bayesian inference has been applied to the analysis of fluctuations of synaptic potentials. The statistical model assumes the synaptic signal to be composed of a mixture of Gaussian components, with unknown means and noise SD (σ). Component attributes include arbitrary spacing and probability; representing synaptic sites at varying electrotonic distances with nonuniform amplitudes. The analysis was applied to minimal EPSPs evoked in CA1 hippocampal neurons. Datasets of peak EPSP values were tested for trends and normality of noise data was confirmed (x² test). Analysis output included: 1) component means and SD values; 2) conditional probability of numbers of components; 3) relative probability of each component; 4) predictive pdf and cdf. A proximal EPSP (n=500, σN=0.18 mV) analyzed to 3 primary components (0.12 mV/22%, 0.21 mV/55%, 0.26 mV/23%) and sums. The predictive pdf/cdf were not different from the raw histogram (x² test: p=0.96). These results were typical for CA1 EPSPs.

The advantages of Bayesian inference over MLE/deconvolution of a mixture distribution include: 1) parameter information can be included in a prior distribution; 2) the conditional probability of the number of components is estimated; 3) the σN value is only an initial estimate of the component variances; 4) the component parameters include uncertainty estimates; and 5) the predictive distributions include variability. However, component separation remains dependent on the σN value, to an extent. Further theoretical work will include more accurate noise description and simulation of known distributions with noise perturbations. Supported by NINDS RO1 NS24602-01 and VAAC.

565.1

THE EFFECT OF MEMBRANE POTENTIAL ON CALCIUM ACTIVATED POTASSIUM CURRENTS. A.R. Martin* and P.A. Fuchs. Dept. of Physiology, Univ. of Colorado Sch. of Med., Denver, CO 80262.

Previous studies have shown that calcium entering though synaptic channels activates calcium-dependent potassium currents (Fuchs and Murrow, J. Neurosci. 12:800-809, 1992). In voltage clamp experiments the potassium currents increased with membrane depolarization and then decreased, approaching zero at about +20 mV, more than 170 mV negative to the calcium equilibrium potential. To account for this unexpected decrease in potassium current at small positive potentials we present a model in which calcium accumulates in a restricted space under the postsynaptic membrane. A second model is also presented to describe the voltage dependence of extrasympathetic potassium currents activated by calcium entry through voltage sensitive calcium channels in chick hair cells (Fuchs, Nagai and Evans, J. Neurosci. 8:2460-2467, 1988). In both models the marked diminution of the potassium currents at positive membrane potentials can be accounted for by (1) an exponential dependence on membrane potential of calcium entry through open channels and (2) a dependence of the potassium current on the fourth power of the intracellular calcium concentration. With these factors the theoretical calculations provide an accurate description of the previous experimental results. (Supported by NIH Grants NS09660 and DC00276).

565.2

ADENOSINE MODULATES A TRANSIENT OUTWARD CURRENT IN RAT LOCUS COERULEUS NEURONS. W. J. Pan* and S. A. Shefher, Dept. of Physiology and Biophysics, University of Illinois, College of Medicine, Chicago, IL 60612.

We have previously shown that adenose decreases the duration of the action potential in locus coeruleus (LC) neurons. In the present study, the mechanism by which adenose affects the shape of the action potential was examined. Intracellular recordings from LC neurons were obtained in a submerged rat brain slice preparation. All drugs were administered by bath application. LC neurons first spontaneously at rates of 1.0 to 3.5 Hz. Ba²⁺ (1 mM) was administered to prolong the duration of the action potential. Adenosine (100-300 μM) reduced the duration of the Ba²⁺ spike measured at 33% of the peak amplitude by 11.5±2.8% (n=7). In these same cells, administration of 4-aminopyridine (4-AP) (30-100 μM) in addition to Ba²⁺ further increased the duration of the action potential. Administration of adenosine then failed to decrease the duration of the Ba²⁺ and 4-AP enhanced action potential. An IA-like transient outward current was investigated using single-electrode voltage clamp in the presence of 0.5 μM TTX and 20 mM Mg²⁺. Inactivation curves were obtained in 5 cells; IA was measured following a 1 sec prepulse to a holding potential between -100 and -40 mV, followed by a 200 ms depolarization to between 0 and +10 mV. This outward current was reduced by 30-100% at a holding potential of 0±1.0 mV, and 30% inactivated at a holding potential of -50±0.9 mV, which is very near the threshold for LC neurons. At threshold, adenosine (300 μM) shifted the inactivation curve in a parallel manner in the positive direction by 4.7±0.5 mV, and increased IA by 132±23%. These data suggest that adenose decreases the duration of the action potential in LC neurons by potentiating an IA-like current. Supported: PHS A005846-09 to S.A.S.
565.5 EFFECTS OF MUSCARINIC AGONISTS ON INTRACELLULAR CALCIUM CHANGES IN ACUTELY DISSOCIATED SYMPATHETIC NEURONS. S. Foscarini and R. J. Miller. Dept. of Pharmacology and Physiological Sciences, University of Chicago, Chicago, IL 60637, USA.

In this study, we evaluated the effects of carbachol (Cch) and oxotremorine (OXO) on intracellular calcium ([Ca^2+]) in neurons acutely dissociated from the superior cervical ganglion of adult rats. The neurons were dissociated enzymatically, plated on poly-l-lysine treated coverslips and used within the next 24 hours. The cells were loaded with Fura-2 AM (3 M, 30 min) and mounted in a perfusion chamber. The perfusion medium contained 2 mM CaCl2, 138 mM NaCl, 5 mM KCl, 2 mM MgCl2, 10 mM HEPES and 10 mM glucose. 

565.7 PATTERN OF CAFFEINE-INDUCED CALCIUM RELEASE IN CULTURED MOUSE HIPPOCAMPAL NEURONS. K. J. Seymour-Laurent* and M. E. Barish. Division of Neuroscience, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The spatial and temporal pattern of Ryanodine-sensitive caffeine-induced calcium release have been investigated using calcium-sensitive dyes (flu-3 and Calcium Green) and confocal microscopy. Mouse hippocampal neurons were dissociated at embryonic day 15-17 and studied after 1-12 days in culture. Cells were continually perfused with normal Hank's BSS containing 0.3 mM CaCl2, 138 mM NaCl, 5 mM KCl, 2 mM MgCl2, 10 mM HEPES and 10 mM glucose. The [Ca^2+]i was estimated by microfluorometry. The basal [Ca^2+]i was 52 ± 3 nM (n=90). Application of Cch (10 μM) or OXO (10 μM) increased the [Ca^2+]i by 86 ± 7 nM and 38 ± 10 nM, respectively. The effects of Cch and OXO were blocked by atropine (1 μM) but not by hexamethonium (10-50 μM). Caffeine sensitive store depletion, peristasis, and closure inactivation and application of staurosporine (1 μM) or thapsigargin (0.1 μM) did not affect the actions of the muscarinic agonists but the option of Ca^2+ from the extracellular as well as Ca^2+ channel block with Ba 3060 (50 μM) abolished it. These results suggest that the increase in [Ca^2+]i produced by muscarinic agonists does not involve the mobilization of intracellular calcium stores, but rather is mediated by Ca^2+ influx through voltage-sensitive calcium channels.


Hippocampal CA1 pyramidal neurons receive cholinergic inputs from septal area. Although this input is thought to play a crucial role in memory, the specific nature of the role is not understood. If cholinergic input is thought to be important in memory, cholinergic agonists might be expected to modify long-term potentiation (LTP) because LTP is believed to underlie memory mechanism. Indeed, it has been reported that cholinergic agonists enhance LTP (Hitzler et al., Shulz & Johnston). Then, what is the mechanism by which cholinergic agonists enhance LTP? Here we report that cholinergic agonists prevent synaptically induced Ca transients from quickly returning back to basal level. We prepared guinea-pig hippocampal brain slices, injected Ca indicator dye fura-2 into a CA1 pyramidal neuron through an intracellular pipette, and monitored intracellular Ca level at its dendrites and the soma while recording membrane potential from the soma. In standard medium, Schaffer collateral synaptic stimulation evoked a transient Ca rise both at dendrites and at the soma. The Ca rise was linked to synaptically evoked action potentials. The Ca level started to decline immediately after the input and quickly recovered to basal level. In contrast, in the presence of 5-10μM carbachol, the decay time of Ca transients became twice as slow as in the standard medium. Although evoked action potentials became wider in carbachol, amplitude of Ca increase driven by supra-threshold synaptic input was not significantly different in both conditions. These facts imply that some secondary processes are responsible for the prolonged Ca transients.

We surmise that prolonged duration of elevated Ca level after conditioning inputs could be the mechanism by which cholinergic inputs enhance LTP. (Supported by MSJC grant 00239105 and HRP).

565.9 Does intracellular Ca^2+ play a role in diameter- or ethanol-induced potentiation of GABA currents in hippocampal neurons? L. Zhang*, J.L. Weiner, A.A. Velumian & P.L. Carlen, Playfair Neurosci. Unit, Toronto Hospital, Depts. of Physiology & Neurosurgery, University of Toronto, Toronto, ON, M5T 2S8. Supported by a Kuffler Fellowship and grants from CONACyT, MRC and NIH.

We studied CA1 hippocampal neurons using whole-cell patch recordings in brain slices. Our standard internal solution contained in mM: EGTA-citrate 130, KCl 20, Mg-ATP 2, GTP-0.5, EGTA 1.1, CaCl2 0.1. In neurons dialyzed with the standard internal solution or an internal solution containing 50 μM EGTA, bath application of 100 μM diazepam, midazolam, or 20 mM ethanol significantly potentiated the GABA-mediated responses. In neurons dialyzed with the internal solution in which EGTA/Ca was replaced by 10 mM BAPTA, the potentiation of GABA-mediated responses by diazepam or ethanol, but not by midazolam, was less pronounced (see graph below). The underlying mechanisms are currently under investigation.


The best known form of synaptic plasticity in cerebellar Purkinje cells (PCs) is a long-term depression (LTD) of excitatory parallel fiber (PF) synapses, resulting from repetitive and conjunctive stimulation of PFs and climbing fibers (CFs). Here we report a new form of synaptic plasticity in cerebellar PCs in which stimulation of the excitatory CF synaptic input leads to a long-lasting (several hours) potentiation of GABA_A receptor-mediated inhibitory postsynaptic currents (IPSCs), a phenomenon which we termed rebound potentiation (RP). By using simultaneous whole-cell patch-clamp recordings and fura-2 video-imaging of intracellular calcium concentration ([Ca^2+]i), we found that a CF-induced transient increase in postsynaptic [Ca^2+]i triggers RP. Several lines of evidence indicate that RP is caused by a Ca-dependent up-regulation of GABA_A receptor function in PCs. The possible role of other second messengers for this up-regulation will be discussed.
### 565.11

**CHANGES IN pH MODULATE NMDA- AND HIGH-[K+] EVOKED RISES IN CYTOSOLIC FREE CALCIUM CONCENTRATION IN RAT HIPPOCAMPAL PYRAMIDAL NEURONS.**

K. Abel-Hamid* and J. Church. Dept. of Physiology and Anatomy, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

NMDA receptor-mediated responses are sensitive to pH changes in [H+]c, which can influence various aspects of neuronal function. This study examined the effects of changes in pH on NMDA-induced increases in [Ca2+]i, using fura-2. All-or-none CF responses were evoked by stimulation of the white matter; IPSPs, blockable by 10 μM bicuculline, were generated by off-beam CNQX superfusion, removal of voltage-dependent block of NMDA channels by withdrawal of Mg2+ (500 μM), and zero Ca media did not affect glutamate-evoked alkalinizations, suggesting that Na channels and Ca-dependent mechanisms do not directly mediate these responses. Dihydrokainate (200 μM) had no effect. During CNQX superfusion, removal of voltage-dependent block of NMDA channels by withdrawal of Mg2+ revealed an APV-sensitive alkalinization similar to that observed in response to intracellular acidification and alkalinization, respectively. The authors suggest that changes in pH might modulate Ca influx via voltage-gated, as well as NMDA receptor-operated, channels.

### 565.12

**NMDA INDUCED REGENERATIVE CALCIUM INFUX IN HIPPOCAMPAL PYRAMIDAL CELLS.**

**O. X. Chen* and R. K. S. Wong.**

Dept. of Pharmacology, SUNY/HSC, Brooklyn, NY 11203.

Whole-cell voltage-clamp was carried out in internally perfused neurons acutely dissociated from the CA1 region of the hippocampus of adult guinea-pigs. Introduction of elevated Ca2+ containing medium into the cell activated an outward potassium current followed by an inward current. The Ca2+-dependent inward current had the following properties: (1) It is activated by intracellular Ca2+ ([Ca2+]i). (2) Its activation is not voltage-dependent. (3) It can be blocked by extracellular cadmium (1 mM). (4) It is in part carried by Ca2+. We tested this inward current Irec+2+. Because of the dependence on [Ca2+]i, and its Ca2+ permeability, we found that Irec+2+ once induced, became self-sustaining and its current level progressively increased following the induction of Irec+2+. Our previous studies show that GABAergic responses in the hippocampal pyramidal cells are suppressed by a dephosphorylation process when [Ca2+]i is elevated (Chen et al 1989, J. Physiol. 420). We now demonstrate that through this mechanism, intense neuronal excitation via NMDA-receptor can suppress GABAergic receptor mediated responses of the same neuron following the induction of Irec+2+.

### 565.13

**NMDA and non-NMDA Receptors Mediate Extracellular Alkalinations in the Rat Hippocampal Slice.**

**T. C. Chen and M. Chest.** Dept. of Physiology & Biophysics and Dept of Neurosurgery, NYU Medical Center, 550 First Ave, N. Y., NY 10016.

Stimulation of Schaeffer collaterals or local application of glutamate evokes picotoxin- insensitive alkalinations in area CA1 (1). We have extended our studies of these responses using pH-sensitive microelectrodes. Ca2+ (100 μM), TTX (1 μM), and zero Ca media did not affect glutamate-evoked alkalinations, suggesting that Na channels and Ca-dependent mechanisms do not directly mediate these responses. Dihydrokainate (100 μM) had no effect on different stimulus-evoked alkalinations, excluding a role for glutamate uptake. In normal media, CNQX (10 μM) blocked Schaeffer collaterals-evoked alkalinations while APV (20-50 μM) had no effect. During CNQX superfusion, responses depend on block of NMDA channels by withdrawal of Mg2+ revealed an APV-sensitive alkalization similar to that observed in response to intracellular acidification and alkalinization, respectively. Individual currents were small (<50 μV at 40 μM and noisy), and in virtual Mg2+ free solution (n=6) showed evident single channel transitions generating irregular shapes. In contrast, activation of other glutamatergic receptors by kainate and QUIS might in part account for the anticonvulsant and neuroprotective effects of cerebral alkalinations in the Rat Hippocampal Slice. J. C. Chen* and M. Chest. Depts. of Physiology and Anatomy, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

### 565.14

**SPONTANEOUS TO EVOKED NMDA CURRENTS IN CEREBELLAR GRANULE CELLS.**


The aim of present work is to compare spontaneous to evoked NMDA current kinetics. Conventional whole-cell recordings of excitatory currents were obtained from granule cells in cerebellar slices at P10-P14. Spontaneous NMDA currents could be observed in the presence of the non-NMDA receptor inhibitor 10 μM CNQX, and were suppressed by 50 μM APV (n=10). Individual currents were small (<50 μA at 40 μM and noisy), and in virtual Mg2+ free solution (n=6) showed evident single channel transitions generating irregular shapes. In contrast, activation of other glutamatergic receptors by kainate and QUIS might in part account for the anticonvulsant and neuroprotective effects of cerebral alkalinations in the Rat Hippocampal Slice. J. C. Chen* and M. Chest. Depts. of Physiology and Anatomy, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

### 565.15

**IPSPs STRONGLY INHIBIT CLIMBING FIBER ACTIVATED [Ca2+]c INCREASES IN THE DENDRITES OF CEREBELLAR PURKINJE NEURONS.**

**J. C. Callaway* and M. Chest.** Dept of Physiology, New York Medical College, Valhalla, NY 10595.

The interaction between IPSPs and climbing fiber (CF) evoked IPSPs was analyzed in the rat cerebellar slice preparation using intracellular recording and high speed fluorescence imaging of intracellularly injected fura-2. All-or-none CF responses were evoked by stimulation of the white matter; IPSPs, blockable by 10 μM bicuculline, were generated by off-beam stimulation in the molecular layer. CF responses generated large, transient [Ca2+]c increases due to Ca spikes. Supported in part by NIH NS12695, NSF BNS-8819188, and a grant from the HFSPO.

### 565.16

**FUNCTIONAL TOPOGRAPHY OF INHIBITION IN RAT SOMATOSENSORY CORTEX.**

**P. A. Salin and D. A. Prince.** Deptartment of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA, 94305.

Postsynaptic inhibition is an important mechanism for regulating neuronal excitability and synchronisation, but the details regarding its laminar distribution are incomplete. We used the whole cell patch clamp technique to record postsynaptic inhibitory currents (IPSCs) mediated by GABAergic receptors in identified pyramidal neurons of layers 2-3 and 5 in adult rat somatosensory cortex (SI). The frequency (3.4-35 Hz) and the amplitude (5-310 μA) of IPSCs were evoked in layer 2-3 and 5 cells when stimulating electrodes were in layer 1. We have extended our studies of these responses using pH-sensitive microelectrodes. Cd2+ (100 μM), and zero Ca media did not affect glutamate-evoked alkalinizations, suggesting that Na channels and Ca-dependent mechanisms do not directly mediate these responses. Dihydrokainate (200 μM) had no effect. During CNQX superfusion, removal of voltage-dependent block of NMDA channels by withdrawal of Mg2+ revealed an APV-sensitive alkalization similar to that observed in response to intracellular acidification and alkalinization, respectively. Individual currents were small (<50 μA at 40 μM and noisy), and in virtual Mg2+ free solution (n=6) showed evident single channel transitions generating irregular shapes. In contrast, activation of other glutamatergic receptors by kainate and QUIS might in part account for the anticonvulsant and neuroprotective effects of cerebral alkalinations in the Rat Hippocampal Slice. J. C. Chen* and M. Chest. Depts. of Physiology and Anatomy, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1Z3.

### 565.17

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**POSTSYNAPTIC MECHANISMS I**

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...potential. If the inhibitory stimulation preceded the CF response by 10-20 msec (the initial phase due to Ca spikes. Supported in part by NIH NS12695, NSF BNS-8819188, and a grant from the HFSPO.
A Low-Frequency Subthreshold Resonance in Neocortical Neurons Generated Mainly by Ih. B. Hutcheon* and E. Puil, *Departments of Physiology and Biophysics, University of British Columbia, Vancouver, BC, Canada. V6T 1Z3

The rhythmic, low-frequency events that characterize the EEG during various behavioral states reflect the coordinated activities of large groups of neurons. We propose that many of these neuronal patterns follow one or more common templates that govern the behavior of whole-cell patch-clamp techniques, the subthreshold responses of neurons in neocortical slices to intracellular injections of oscillatory inputs in order to determine membrane impedance as a function of frequency. The neurons exhibited properties that bias their responses to low frequencies (< 10Hz).

The magnitude of the impedance in some neurons declined steeply and monotonically, with frequency (1/2 magnitude, 5-20 Hz). In other neurons, the impedance magnitude increased with frequency, and the center of gravity (the centroid) of the resonant hump did not depend on the time course of the input function, or whether current or voltage was used as input. Modeling of Ih and the absence of other slow voltage-gated currents such as If showed that Ih by itself is capable of producing the resonant behavior in neocortical neurons.

INTEGRATIVE PROPERTIES OF HIPPOCAMPAL, CORTICAL AND THALAMIC NEURONS STUDIED BY WHITE NOISE ANALYSIS. H. Jahnsen* and S. Karnup Institute of Neurophysiology, Blegdamsvej 3 c, DK-2200 Copenhagen N, Denmark.

The signal delay of neurons is significantly affected by the cable properties and morphology of the dendrites. This dendritic delay plays an important role in information processing and in plastic processes at the neuronal level. A novel analytical approach for calculating the delay of electrical signals in any passive dendritic tree is introduced. The dendritic delay (D, Delay) is defined hereby as the difference between the centroid of the resonant current input and the center of gravity of the resultant voltage transient, evaluated at any point in the tree. The D, Delay measured at the input point is non-zero and is called the local delay (L, Delay). The D, Delay is defined hereby as the difference between the centroid of the resonant current input and the center of gravity of the resultant voltage transient, evaluated at any point in the tree. The D, Delay measured at the input point is non-zero and is called the local delay (L, Delay). Propagation delay (P, Delay) is defined hereby as the difference between the centroid of the resonant current input and the center of gravity of the resultant voltage transient, evaluated at any point in the tree. The D, Delay measured at the input point is non-zero and is called the local delay (L, Delay).

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POSTSYNAPTIC MECHANISMS I

DIFFERENCES IN REPEETITIVE FIRING BETWEEN ADULT AND IMMATURE RAT SENSORIMOTOR CORTICAL NEURONS MIMICKED BY CALCIUM CHELATION. Lov螸en, N. M* and Foehring, R. C. Dept of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38133-4901.

In adult cortical pyramidal cells, long current injections result in regular, repetitive firing followed by a slow afterhyperpolarization (AHP). In immature (P6-10) neurons, the AHP follows the burst discharge and is greatly prolonged relative to adult cells, resulting in more prominent spike-frequency adaptation in the immature cells. Some immature neurons will fire not longer than 200 ms. Several potential mechanisms could explain these age-dependent differences. One possibility is that Ca2+-handling differs at the two ages. A recent study in cat motor cortex (Schwindt et al. 2001) suggested that low doses of the Ca2+ chelator BAPTA were included in the intracellular electrode. We tested whether the immature firing pattern could be elicited in adult rat cortical neurons impaled with electrodes including 2 mM BAPTA (in 2 M K-methylsulphate), and whether higher doses of BAPTA (200 mM) resulted in loss of the AHP and spike-frequency adaptation in young and mature neurons. Intracellular recordings were obtained from an in vivo slice preparation.

We found that 200 mM BAPTA greatly reduced AHPs and adaptation at both ages. 2 mM BAPTA caused adult neurons to have enhanced, prolonged afterhyperpolarizations, and the immature firing pattern. The BAPTA-enhanced AHP in adults was reduced by 100 µM isoprotroline and when extracellular Ca2+ was replaced with Mg2+.

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Supported by ONR and NIH.

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POSTSYNAPTIC MECHANISMS II

SPACE-CLAMP ERRORS ASSOCIATED WITH MEASUREMENT OF ELECTROTONICALLY REMOTE SYNAPTIC EVENTS. N. Spruston*, D. Jaffe, S. H. Williams and B. D. Johnston, Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The frequency-dependent voltage attenuation and resultant space-clamp errors associated with the measurement of synaptic current are evaluated both in equivalent circuit models and in a detailed compartmental model of a hippocampal CA3 pyramidal neuron. We show that in both models, as expected, higher-frequency voltage changes are attenuated to a much greater extent than steady-state or low-frequency voltage changes.

The effect of increases in membrane resistivity (Rm) were also examined and found to dramatically reduce steady-state voltage attenuation, while having only small effects on the non steady-state components of synaptic responses. The consequence of the frequency-dependent reduction in voltage attenuation by increases in Rm is that exponentially increasing Rm (e.g., with the use of Ca2+-containing patch-clamp electrodes) can result in improvements in the accuracy of measured reversal potentials (which are in error according to the electrotonic distance of the synapse) without substantial improvements in the accuracy of peak current, conductance, rise time, or decay time constant measurements (which are in error according to the electrotonic distance for the high-frequency components in fast synaptic responses).

Finally, we use simulations from a morphological model of a CA3 pyramidal neuron to demonstrate the distortion of mossy fiber and commissural/associational synaptic currents at the soma, and to estimate the electrotonic distances of mossy fiber and presynaptic path synapses. We conclude that the error associated with synaptic current measurement can be extremely large for synapses located at electrotonically remote sites in the dendritic tree. (Supported by MH4757, MBR84631, the Kroc Foundation, and the A. von Humboldt Foundation.)

565.5

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Single Neuron Computation, R. L. Jaffe and J. Brown, Soc. Neurosci. Abstr. 18, in press). Three principal conclusions emerged from these simulations. First, thorns have essentially no effect on transfer of potential, current or charge from spine head to soma, or from spine head to spine head. Second, the network might be used to demonstrate the sensitivity of whole-cell voltage clamp recordings so that synaptic reversal potential, charge transfer, peak conductance and peak current can be determined accurately for many MF inputs. Third, MF synapses are not "demons"—at least 10 must be activated to trigger a somatic spike. Supported by ONR and NIH.
PRESYNAPTIC MECHANISMS II

POSTSYNAPTIC MECHANISMS II

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with the new values to get comparable action potentials. The DAP following a

of Neuropharmacology, The Scripps Research Institute, La Jolla CA,

granule cells, studies with perforated-patch electrodes (Spruston and

was smaller, and consequently, calcium influx through NMDA receptor

channels was smaller with the old Rm and Ri values than with the new ones.

It might be possible to compensate for some of these differences by changing conductance kinetics, or by adding additional conductance to limit the number of degrees of freedom, one must fix appropriately and accurately as many parameters as possible, beginning with Rm and Ri.

566.7

PREPROPENEOL SULFATE EFFECTS UPON CA1 HIPPOCAMPAL NEURONS IN A SLICE PREPARATION. J.H. Meyer * and D.L. Gruol. Dept.

PREGNENOLONE SULFATE EFFECTS UPON CA1 HIPPOCAMPAL

conductance parameters were adjusted to give comparable peak EPSPs with

both sets of Rm and Ri. When synapses were activated at 100 Hz, the peak

potential was larger (due to action potential inversion), but average potential was smaller, and consequently, calcium influx through NMDA receptor

channels was smaller with the old Rm and Ri values than with the new ones.

566.8

A SYSTEM FOR THE ELECTROSTATIC STIMULATION AND MEASUREMENT OF c-fos AND PPT GENE EXPRESSION IN RAT SUPERIOR CINGULAR CEREBRIAL NEURONS USING THE MID-DENDRITIC REGION WAS MODELLED. Synaptic activity in the mid-dendritic region was modeled. Synaptic conductance parameters were adjusted to give comparable peak EPSPs with both sets of Rm and Ri. When synapses were activated at 100 Hz, the peak potential was larger (due to action potential inversion), but average potential was smaller, and consequently, calcium influx through NMDA receptor

channels was smaller with the old Rm and Ri values than with the new ones.

566.9


To assess the role of nitrogen-activated protein kinase (MAPK) in neuronal systems, we have examined its localization in brain immunohistochemically and its regulation by neurotransmitters in primary cortical cultures. Light-microscopic studies reveal prominent staining of neuronal cell bodies and dendrites, particularly in the hippocampal CA3 region and dentate gyri, pyramidal cells in the cortex, and Purkinje cells in the cerebellum. In the ultrastuctural level, intense staining is localized to dendritic microtubules, as well as the Golgi apparatus.

566.10

ASSEMBLY AND PKC-PHOSPHORYLATION OF RC3 (NEUROGRANIN) IN RAT BRAIN SYNPATOMES AND VOLTAGE-CLAMPED XENOPUS OOCYTES. P.M. Coulter II, R.W. Cohen, J.W. Margulies and J.B. Watson. Mental Retardation Research Center and Dept. of Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA 90024.

RC3 (neurogranin) is a forebrain-enriched 78 amino acid protein containing overlapping sites for protein kinase C (PKC) phosphorylation and calmodulin binding that are shared with GAP-43. In contrast to GAP-43's presynaptic localization, RC3 accumulates primarily in dendritic spines of forebrain neurons. Here we use combined biochemical and immunohistochemical methods to examine RC3's state of assembly and PKC-phosphorylation in brain synaptosomal fractions. Conventional electron-physiological studies of RC3 heterogeneous oocytes expressed in Xenopus oocytes test the hypothesis that phosphorylated RC3 has a function in PKC-activated signal transduction pathways. Western blot analysis indicates that RC3 mRNA expression in oocytes are resistant to most biochemical treatments but are solubilized by non-ionic detergents (triton X-100, sarcoside), guandihydrochloride, and alkaline conditions. We conclude that RC3 is tightly associated with synaptosomal membranes presumably through ionic interactions with postsynaptic membranes. Immunoprecipitation experiments (in progress) may reveal linkage between RC3 expression and synaptic activity. Voltage-clamp experiments: foliated Xenopus oocytes expressing RC3 show enhanced responses to acetylcholine (2-3 fold), measured as calcium-activated Cl- currents. RC3 enhanced acetylcholine responses are dampened to control levels by the PKC- inhibitor H7. Phorbol ester-treated oocytes expressing RC3 exhibit significant calcium/chloride currents that are not observed in control oocytes. The cumulative data suggest that phosphorylated RC3 modulates calcium ion levels in dendritic spines of forebrain neurons.

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RAT PINEALOCYTES: NOREPINEPHRINE INDUCES DESENSITIZING Ca2+ RELEASE FROM INTRACELLULAR STORES AND NONDESENSITIZING Ca2+ INFLUX FROM THE MEDIUM. J.C. Sánchez, Spain.

Synthesis and secretion of melatonin by mammalian sympathetic neurons and respond to norepinephrine with a rise in intracellular Ca2+ (see abstract by Sánchez, et al.). Previous studies have shown that these cells are electrically coupled by gap junctions, and that connexin26 (Cx26) is the primary gap junction protein. Using a dual whole cell voltage clamp method with patch-type electrodes, we have characterized the physiological properties of pinealocyte gap junction channels after dissociation of cell pairs. 25% of the cell pairs studied showed electrical coupling, with junctional conductance values between 200 pS and 2 nS. The unitary conductances of the junctional channels were 40-50 pS in some cell pairs simultaneous openings of clusters of channels were recorded, suggesting a novel type of cooperativity between the channels. Voltage dependence of macroscopic junctional conductance (gj) was slightly asymmetric; whereas transjunctional voltages (Vj) of both signs decreased gj, the Vj value at which the voltage sensitive component of gj is reduced by 50% was about ± 40 mV, and the equivalent gating charge was ± 0.3 for depolarization and about 0.5 for hyperpolarization. These channel characteristics differ from those seen for other connexins expressed in other tissues, therefore providing additional evidence that the junctional protein may be connexin26.


Pinealocytes are neural secretory cells found in the pineal gland that are responsible for the synthesis and release of melatonin. They are postsynaptic to sympathetic neurons and respond to norepinephrine with a rise in intracellular Ca2+ (see abstract by Sánchez, et al.). Previous studies have shown that these cells are electrically coupled by gap junctions, and that connexin26 (Cx26) is the primary gap junction protein. Using a dual whole cell voltage clamp method with patch-type electrodes, we have characterized the physiological properties of pinealocyte gap junction channels after dissociation of cell pairs. 25% of the cell pairs studied showed electrical coupling, with junctional conductance values between 200 pS and 2 nS. The unitary conductances of the junctional channels were 40-50 pS in some cell pairs simultaneous openings of clusters of channels were recorded, suggesting a novel type of cooperativity between the channels. Voltage dependence of macroscopic junctional conductance (gj) was slightly asymmetric; whereas transjunctional voltages (Vj) of both signs decreased gj, the Vj value at which the voltage sensitive component of gj is reduced by 50% was about ± 40 mV, and the equivalent gating charge was ± 2.5, the proportion of gj remaining at high voltages was about 0.3 for depolarization and about 0.5 for hyperpolarization. These channel characteristics differ from those seen for other connexins expressed in other tissues, therefore providing additional evidence that the junctional protein may be connexin26.

In the projection from prefrontal cortex to nucleus accumbens (Acb), recordings were made in parasagittal slices (400 µm thick) from male Wistar rats. Stimulus electrodes were positioned at the border between infralimbic cortex and Acb. Picrotoxin (10 µM) was present in the bathing medium. Resting membrane potential, input resistance and action potentials were recorded in the absence of glutamate iontophoresis. In the presence of 100 µM picrotoxin and the rate of rise of field EPSPs was recorded for a control condition with 2 mM CaCl2. Following washout of kynurenic acid, this protocol was repeated with 20 µM AP5, the same conditioning protocol produced a similar enhancement of EPSC amplitude (mean increase 80%) lasting at least 15 minutes. When the high-frequency stimulation, iontophoresis was performed in medium containing 2 mM CaCl2, AP5, and the bath contained 20 µM AP5, the same conditioning protocol produced a similar enhancement of EPSC amplitude (mean increase 65%) in 16/46 cells, and this potentiation could be maintained for at least 1 hr. Preliminary results indicate that AP5-resistant potentiation is specific to the synapses that were active during posttreatment depolarization. Thus, NMDA receptor activation is not necessary for the induction of LTP in CA1 pyramidal cells by pairing when using the whole-cell recording technique.


567.3


The simultaneous activation of pre- and postsynaptic elements, and an accompanying rise in intracellular Ca2+ concentration, is a necessary condition for LTD induction. We have tested the hypothesis that simultaneous (Hebbian) rises of pre- and postsynaptic Ca2+ are sufficient to induce LTP in the absence of fast glutamatergic and GABAergic neurotransmission. Extracellular recordings were done in the str. radiatum of the CA1 region of hippocampal slices at 34-35°C. Schaffer collateral-commissural fibers were stimulated once every 30 s in the presence of 100 µM picrotoxin and the rate of rise of field EPSPs was recorded for a control condition with 2 mM CaCl2. Following washout of kynurenic acid, this protocol was repeated with 20 µM AP5, the same conditioning protocol produced a similar enhancement of EPSC amplitude (mean increase 80%) lasting at least 15 minutes. When the bath contained 20 µM AP5, the same conditioning protocol produced a similar enhancement of EPSC amplitude (mean increase 65%) in 16/46 cells, and this potentiation could be maintained for at least 1 hr. Preliminary results indicate that AP5-resistant potentiation is specific to the synapses that were active during posttreatment depolarization. Thus, NMDA receptor activation is not necessary for the induction of LTP in CA1 pyramidal cells by pairing when using the whole-cell recording technique.


567.4

NMDA RECEPTOR ACTIVATION AT SYNAPSES ON CA1 NEURONS IS NOT NECESSARY FOR THE INDUCTION OF LTP. A. C. Field, S. J. Redman and C. Strockter Division of Neuroscience, JCSMR, Australian National University, Canberra, ACT 2601, Australia.

The induction of LTP at synapses on hippocampal CA1 neurons is believed to occur as a result of calcium influx through NMDA channels, since it is prevented by the NMDA receptor antagonist 2-amino-5-phosphono-pentanoic acid (AP5). However, an AP5-resistant component of LTP has been observed when the rate of tetanic stimulation of presynaptic fibers was increased to 200 Hz (1). We have investigated the AP5-sensitivity of LTP induced by pairing low frequency (2 Hz) activation of small groups of afferents with depolarization of the postsynaptic neuron to 0 mV, under whole-cell voltage clamp conditions and with calcium-based intracellular solutions, in CA1 pyramidal cells in 400 µm rat hippocampal slices. In the absence of AP5, an enhancement of EPSC amplitude (mean increase 80%) lasted at least 15 minutes was observed in 14/26 cells following pairing. When the bath contained 20 µM AP5, the same conditioning protocol produced a similar enhancement of EPSC amplitude (mean increase 65%) in 16/46 cells, and this potentiation could be maintained for at least 1 hr. Preliminary results indicate that AP5-resistant potentiation is specific to the synapses that were active during posttreatment depolarization. Thus, NMDA receptor activation is not necessary for the induction of LTP in CA1 pyramidal cells by pairing when using the whole-cell recording technique.


567.5


In this study we examined what types of plastic changes can be found in the projection from prefrontal cortex to nucleus accumbens (Acb), a structure implicated in reward-dependent learning. Intracellular recordings were made in parasagittal slices (400 µm thick) from male Wistar rats. Stimulus electrodes were positioned at the border between infralimbic cortex and Acb. Picrotoxin (10 µM) was present in the bathing medium. Resting membrane potential, input resistance and action potential amplitude of the recorded neurons (N=75) were 78 ± 2 mV, 42 ± 14 mV and 93 ± 5 µA (50 Hz, 2 sec) in CA1 pyramidal cells by pairing when using the whole-cell recording technique.

567.6

LONG-TERM POTENTIATION OF PERFORANT-PATH INPUT TO CA3 PYRAMIDAL NEURONS IN HIPPOCAMPAL SLICES. S.-F. Chot* B. D. Johnston, Dept. of Molecular Physiology and Biophysics. & Div. of Neuroscience, Baylor Col. of Med., Houston, Tx 77030.

Direct projections of perforant path (PP) fibers from entorhinal cortex to the hippocampal area CA3 have been demonstrated anatomically but their physiological properties have only recently been investigated (Yezzi and Berger, 1990, Proc. Natl Acad. Sci. USA 87:5382-5386). As a prelude to further study of the cellular events associated with dendritic inputs, we examined by field recording and lesion methods whether direct PP input to CA3 can be selectively activated and whether action potentials and LTP can be evoked by this input in the rat hippocampal slice preparation.

In slices with part of CA1 and DG removed, paired-pulse facilitation could be recorded in S. lacunosum-moleculare (SLM) and S. pyramidale of CA3 by stimulating remnant SLM of fimbria or residual SM of DG (400 µm). The latencies of the pEPSPs were 10 ms, which is consistent with a monosynaptic response. LTP of the PP termination was not prevented by NMDA receptor antagonists, but the LTP was blocked by APV (100 µM). These results suggest that the PP has functional monosynaptic input to CA3 pyramidal neurons via SM of both CA1 and DG is the site of LTP induction.

567.1


The impact of probabilistic transmissions on the input-output characteristics of individual neurons has received some theoretical, but little experimental evaluation. If action potential generation requires the summed input above a large percentage of the 1000 mV synapses on an average CNS neuron, the total response will show little inter-trial variation even though individual synaptic events are variable. In this study, we have recorded postsynaptic responses in hippocampal neurons given a constant afferent stimulus that recruits sufficient presynaptic fibers to produce a postsynaptic EPSP. We note a significant inter-trial variability that is of synaptic origin and can be modulated by physiological manipulation. Two identified cell populations have different variability at threshold. Theoretical considerations suggest that inter-trial variability will have different effects on trial-to-trial variability. We find a decrease in inter-trial variability associated with LTP. If postsynaptic response is reduced by recruiting fewer fibers, the trial-to-trial variability returns to that observed before potentiation. We thus observe maintained variability of the input-output relations despite the large gain changes observed with LTP.
L-TYPE CALCIUM CHANNELS ARE INVOLVED IN THE INDUCTION OF NMDA RECEPTOR-DEPENDENT LONG-TERM POTENTIATION (LTP) IN ADULT RAT HIPPOCAMPUS. A. Villahermosa, N. Koutsoukos, L. Angelopoulos, T. I.Tyler and C. Stefani. Univ. of Athens, Dept. of Psychiatry, Eginition Hospital, 11528 Athens, Greece and Northeastern Ohio Coll. of Med., Rootstown, OH 44272 U.S.A.

In visual cortical slices from Mistar rats (age 60-80 days) the field potentiation in layer III elicited by white matter stimulation consisted of a negative component (NI) with peak latency 4-8 msec, which was unaffected by bath applied APV. In a few slices a second component was present (peak 12-19 msec) which was insensitive to APV in most of these slices. Bath applied DNX blocked NI and revealed the presence of an APV-sensitive component (peak 9-20 msec). Tetanic stimulation in normal medium induced LTP (138-385%) of NI in 72% of the slices. The presence of APV, LTP of NI (145-279%) was induced in 75% of the slices. Potentiated responses were unaffected by APV. Bath application of the dihydropyridine antagonist nifedipine did not affect control responses. Following tetanic stimulation in the presence of nifedipine no change or long-term depression was observed in 80% of the slices, while a small magnitude LTP (130-150%) was induced in the remaining slices. The known reduction of NMDA receptor activity with age is accompanied by an increased importance of voltage-gated Ca++ channels in maintaining synaptic plasticity.

EFFECT OF TRIMIPRAMINE, AN ANTIDEPRESSANT, ON HIPPOCAMPAL SYNAPTIC PLASTICITY. G. Massicotte, M. Ohayon AND J. Bernard. Lab. of Neurobiology, Université du Québec à Trois-Rivières, Québec, Canada, G9A 5H7.

The effect of trimipramine (TRIM), an antidepressant agent, on both the induction and the maintenance of long-term potentiation (LTP) was investigated in field CA1 of hippocampal slice preparation. Chronic administration (7-9 days) of TRIM in rat caused a large reduction in the magnitude of LTP induced by theta burst stimulation (TBS) paradigm. Trimipramine had no significant effect on either the degree of facilitation in postsynaptic responses occurring during TBS or the amount of paired-pulse facilitation. Furthermore, this facilitation of post synaptic responses occurring in the first two minutes following the high frequency stimulation was not reduced by trimipramine administration. These results indicate that TRIM interferes with the formation of LTP, an effect that is not due to alteration of physiological event that triggers LTP. The data suggest that the loss of LTP maintenance is more likely the result of the disruption by trimipramine of cellular processes that follow LTP induction. In addition, the present results provide evidence for a possible correlation between reduction in LTP expression and learning deficit produced by chronic administration of trimipramine.

This work was supported by NSERC of Canada.


Previous studies have shown that LTP in layer III of visual cortex is difficult to induce and of a limited size (in terms of percent change in amplitude of field potentials) in adult rats (Perkins & Tyler, 1988; Kabe et al., 1991). Moreover, seemingly identical stimulation regimes may induce either short-term depression (STD) or LTD (Berntson et al., 1988). Here, we present a new form of LTP in adult rat visual cortex. In our experiments, extracellular recording electrodes were placed in layer V/VI of rat visual cortex, while stimulating electrodes were located in the white matter layer of the recording electrode. Evoked responses were examined only if population spike spikes (PS-like spike, onset latency from 3 ms to 7.5 ms), EPSP-like potentials (peak before 12 ms) and long-lasting inhibitory responses following EPSP-like potentials were present. We found that the magnitude of PS-like potentials was increased by white light to about 200% (some times more) of control levels after tetanus in most cases. In many cases the onset latency of PS-like spikes was shortened after tetanus. In contrast, the sizes of PS-like potentials and PS-like spikes were increased by white light, and decreased by white light (1 case) or an increase (1 case). Thus, there is a mismatch between LTP-like potentials and PS-like spikes during cortical LTD. Many cases could be viewed as indicating LTD if the really EPSP-like potentials were measured. If the PLike spikes is considered, we find that there is still a substantial apparent induction of LTD. Because of this mismatch, the measurement of PS-like spikes may be a more sensitive measurement of LTD in visual cortex. Supported by MH33231 and NSF BMS-912129

Dexamethasone blocks the induction of hippocampal primed burst potentiation. David M. Diamond*, Bernlyn Branch, M. Catherine Bennett, Monika Fleshner and Gregory M. Rote. Department of Pharmacology, UCHSC and VAMC, Denver, CO 80262-2115.

There is a negative correlation between corticosterone (CORT) levels and PB potentiation, a form of long lasting hippocampal plasticity induced by physiologically patterned stimulation (Psychobiol., 19:301, 1991). In the present study, we investigated the possibility that this effect was mediated by the Type II glucocorticoid receptor.

We stimulated the hippocampal commissure and recorded CA1 population spikes in urethane-anesthetized adrenalectomized rats. PB potentiation (5 pulses: a priming pulses followed 170 ms later by a burst of 4 pulses at 200 Hz) was delivered 3-4 hours after administration of dexamethasone phosphate (DEX; 300-900 µg, i.p.), a selective Type II agonist. A dose-dependent inhibition of the incidence and magnitude of PB potentiation by DEX was observed. PB depression, a lasting decrease in the amplitude of the population spike, occurred at the 900 µg dose. PB depression was originally identified in animals with high levels (> 60 µg/dl) of CORT. Therefore, DEX mimicked the effects of elevated levels of CORT.

These findings suggest that occupation of the glucocorticoid Type II receptor results in inhibition of hippocampal plasticity. Our observations imply a role for CORT in mediating the effects of stress on hippocampus-dependent learning.


Previous research has demonstrated that application of β-adrenergic agonists in vitro can produce long-lasting potentiation (LLP) of field responses evoked in the dentate gyrus (DG) by stimulation of the medial perforant path (PP) and long-lasting depression (LLD) of responses evoked by stimulation of the lateral PP (Dohl & Surveys, JNS, 1989; Pelletier, Kirkby & Corcoran, Soc. Neurosci. Abstr., 1991). We report here that β-adrenergic blockade produces LLP of responses evoked by stimulation of the medial PP and LLD of responses evoked by stimulation of the lateral PP.

Transverse hippocampal slices from male hooded rats were placed in an interface chamber, and field potentials were recorded from the outer or middle molecular layer. After stable baseline responses were obtained, the β-adrenergic antagonist (-)-metoprolol (-)-sulfate (MET; 20 µM) was bath applied for 30 min, followed either by a 30 min wash and tetanization (TET) or by a 60 min wash with no TET.

MET produced LLP and LLD of responses evoked by stimulation of the medial PP and the lateral PP, respectively, that persisted for the 60 min wash. TET of the medial PP potentiated evoked responses back to baseline levels, above the depression produced by MET. TET of the lateral PP produced additional potentiation above that produced by MET. These results provide further evidence for the pathway specificity of β-adrenergic plasticity of responses evoked in the DG, and they also suggest that antagonism of β-adrenoceptors has long-lasting effects on synaptic transmission in the DG.

EFFECTS OF BURST STIMULATION ON NEIGHBORING SINGLE CA1 NEURONS IN RAT HIPPOCAMPUS. P.D. Marin, N. Lake*, M.L. Shapiro. Depts. of Psychology & Physiology, McGill University, Montreal, Quebec, Canada, H3A 1B1.

The effects of burst stimulation (ten 25 ms bursts of 400 Hz pulses at 200 ms intervals) on single CA1 neuron activity were recorded with stereoelectrodes placed in the pyramidal layer of CA1 and stimulating electrodes implanted in the contralateral CA3 of urethane anesthetized rats. Individual units were distinguished by waveform parameters, and the firing pattern of each unit was analyzed separately. The latency of evoked unit firing was commensurate with the latency of evoked field potentials. (Four of nine discriminated cells fired spontaneously. Five cells fired upon stimulation, and no cells fired both spontaneously and upon stimulation. Burst stimulation increased field EPSP amplitude (>-30 min) and decreased spontaneous firing in three of four spontaneously firing cells. Of the five cells that displayed evoked firing, burst stimulation increased the firing probability in one cell, reduced the firing probability in one cell, and had no effect on the other three cells. Thus, neighboring cells recorded simultaneously were affected differently by burst stimulation. Changes in both feed forward excitatory and inhibitory connections may contribute to the pattern of altered neural responses produced by burst stimulation and LTP induction.)
**LONG-TERM POTENTIATION OF THE IPSILATERAL-ASSOCIATIONAL PATHWAY IN THE RAT DENTATE GYRUS.**


Mossy fibers from granule cells in the dentate gyrus of the rat hippocampal probe to mossy cells in the hilar region, which in turn make excitatory synapses on the apical dendrites of the granule and dentate gyrus. To relate physiological and anatomical properties of this excitatory feedback system, multiple electrodes (100µ, tungsten rod) were located along the longitudinal axis of the dorsal lateral geniculate nucleus (LGN) and recorded in the unanesthetized rat (2.0 kG), Long Evans). Single pulses delivered to the hilar region evoked negative-going, mono-synaptic electrical field potentials (EPFs) in the ipsilateral first-order nigrostriatal pathway. These EPFs were recorded simultaneously at four locations, the peak amplitude of which varied linearly with distance from point of stimulation, and could be elicited either rostral or caudal to the stimulating electrode. A stimulus train (ten 25 ms bursts of 400 Hz pulses at 200 ms intervals) was delivered to the hilar region, and the initial slopes of the recorded EPF's were potentiated (21%). Potentiation lasted at least two hours and was specific to responses from the tetanized stimulating electrode; the responses to a second stimulating electrode in the hilar and a third in the angular bundle of the perforant path did not change. The results suggest that the ipsilateral association system of the dentate gyrus supports long-lasting and synapse-specific changes in electrical response.

**LTP CHANGES THE WAVEFORM OF SYNAPTIC RESPONSES: PROXIMAL VERSUS DISTAL APICAL DENDRITIC SYNAPSES.** A. Kelso, P. Xiao, & G. Lynch. CNLM, Univ. of Calif., Irvine, CA 92717.

In a previous study, we demonstrated that long-term potentiation (LTP) was accompanied by a decrease in the decay time constant of excitatory synaptic field potentials in field CA1 of hippocampus, suggesting that potentiation alters the means open time of AMPA type glutamate receptors. In the present study, we compare field potentials evoked by stimulation of axons at proximal and distal levels of the apical dendritic tree, and measured waveform parameters of responses recorded at corresponding locations during paired pulse facilitation (PPF) and LTP. Exocytotic responses in minislices of field CA1 were elicited by blocking GABAergic responses with picrotoxin and 2-hydroxyaclofen. Decay time constants (DTCs) were measured by exponential fitting. LTP was induced by theta burst stimulation and PPF was measured at inter pulse intervals of 85 ms.

Under control conditions, responses were larger when recorded locally (at the same proximal-distal position as the stimulation), indicating that the stimulation electrodes activated spatially distinct synapses. DTCs were longer for responses recorded proximally, regardless of stimulation locus. PPF had no effect on DTCs; however, LTP significantly reduced the DTC for all responses. LTP was larger when recorded proximally, regardless of stimulation position; PPF was not affected by either stimulation position or recording position.

Comparison of PPF with LTP provides further support for the hypothesis that LTP involves changes in postsynaptic receptors. PPF did not change response DTCs whereas LTP did. Differences between proximal and distal synapses suggest spatial variations in LTP induction and/or expression mechanisms. (Supported by ONR #N00014-89-J-1255 and FRSQ and NSERC of Canada.)

**FACTORS REGULATING THE MAGNITUDE OF LONG-TERM POTENTIATION INDUCED BY THETA PATTERN STIMULATION.** A. Aarón and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717-3800.

Electrical stimulation patterned after the hippocampal theta rhythm produces robust and stable long-term potentiation (LTP). The present study was designed to examine responses to different parameters of the theta rhythm and to characterize the neural substrates of LTP. We studied the induction of LTP at various patterns of theta rhythm, including theta pattern stimulation (TPS) and paired pulse facilitation (PPF). TPS was effective in inducing LTP, while PPF had no effect. The relationship between theta frequency and LTP was complex, with an optimum frequency of 8 Hz. LTP was also induced by theta burst stimulation, but only when the burst frequency was 12 Hz or greater. The results suggest that the LTP induction is mediated by a synaptic mechanism that is specific to the theta rhythm.

**PRIMING OF ASSOCIATIVE LTD IN THE DENTATE GYRUS BY THETA-FREQUENCY SYNAPTIC ACTIVITY.** B.R. Christie *and W.C. Abraham. Department of Psychology and the Neuroscience Research Centre, University of Otago, Dunedin, New Zealand.

The present study evaluated the role of (NASS) LTD in the induction of LTP by theta rhythm. Theta rhythm was induced by stimulating the dentate gyrus with a pattern of theta rhythm for a duration of 5 Hz. The results indicated that theta rhythm was effective in inducing LTP, but only when the theta rhythm was presented immediately before or after the theta rhythm. The results also suggest that theta rhythm is a necessary but not sufficient condition for the induction of LTP.
567.19

INPUT ASYNCHRONY PROLONGS THE RISING PHASE OF MOSSY FIBER-EOVOKED EPSCS IN RAT HIPPOCAMPAL CA3 PYRAMIDAL CELLS. R. B. Landgraf, J. W. Johnson, and D. Barretelle. Dept. of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260

Investigation of LTP at mossy fiber (MF) synapses depends upon discrimination of monosynaptic, MF-EOVoked EPSCs (MF EPSCs) from higher order EPSCs, based upon expectation for MF EPSC kinetics. Using whole-cell recording, we have examined EPSCs evoked by stimulus applied to the s. granuleum. Rise-time durations ranged from 0.7 to 5.0 msec (mean = 2.5 msec; N = 27). Most rising phases were inflected, implying asynchronous arrival of inputs. Hypothetically, this could be due to "contamination" via activation of local axon collaterals. If so, that generalized suppression of neurotransmission should alter not only the amplitude but also the shape of EPSCs. We found no CNQX + APV, or low [Ca(2+)](high) mGluR(1) medium (using a subthreshold-type slice chamber) did not shorten long rise-times or remove inflections (N = 5; amplitudes were reduced 64 to 88%.)

We examined the shape of MF impulse activity by recording (from slices in medium that blocked neurotransmission) the extracellular potential generated by action currents in MFs (the MFV). Typical MFVs lasted 3 to 4 msec, with long and complex negative phases, implying impulse asynchrony (N = 13). This temporal dispersion could be the result of (hypoth.) dispersion of MF conduction velocities, (hypoth.) dispersion of conduction distances, or (hypoth.) unconventional MF physiology. We dismiss hypoth. I because antidromically activated granule cell population spikes broadened only slightly as a function of distance between stimulating and recording sites. Hypoth. II would contribute to asynchrony if impulses traveled anti- dromically in hilar MF collaterals, then orthodromically in MFs. However, lesions that would interrupt such impulses did not produce brief, simple (triphasic) MFV waveform. Therefore, long and inflected MF EPSC rising phases may be due to intrinsic physiology of MF axons or their boutons. Supported by NS34284 and MH51566.

567.21


We have developed a maximum likelihood (ML) approach to quantify uncertainty within the context of several testable models. Systematic Monte Carlo simulations amplify the model's power and justify the application of ML theory to each specific data set. These methods of quantal analysis were applied to rat hippocampal mossy-fiber (mf) synapses, which offer a number of advantages for studies of synaptic microstructure (physiology) (Siegel et al., Soc. Neurosci. Abstr. 18, in press; Yu and Brown, Soc. Neurosci. Abstr. 18, in press; Jaffe and Brown, Soc. Neurosci. Abstr. 18, in press; Xi an et al., Soc. Neurosci. Abstr. 18, in press). Whole-cell voltage-clamp recordings were made of evoked currents that satisfied appropriate criteria for mf origin (Xi an et al., Soc. Neurosci. Abstr. 18, in press).

In a study of mf-paired-pulse facilitation, a mf input was stimulated twice in rapid succession for several hundred trials. For various measures, the first and second response amplitude distributions could be well-fit by a Poisson or binomial probability function for quantal release and a gamma or Gaussian probability density function for quantal size. The mean quantal size did not increase with facilitation. Facilitation resulted, instead, from an increase in the mean quantal content. We are currently investigating the quantal basis of long-term potentiation in mf synapses. Supported by NIH and ONR.

567.23

HEBBIAN LEARNING IS JOINTLY CONTROLLED BY ELECTROTONIC AND INPUT STRUCTURE. B.A. Pearlmutter1 and T.H. Brown2. Departments of Psychology and Cellular & Molecular Physiology, Yale Univ., New Haven, CT 06520.

Hebbian learning in a linear isopotential neuron with instantaneous response has been shown to cause the synaptic weights to tune to the principal eigenvector of the instantaneous input correlation matrix Q. Q = xi j (Amari 1977 Biol. Cybern. Oja et al. 1985 J. Math. Anal. Appl.) Here, we show that in the nonisopotential case, the important matrix is Q = (Qij)m×n = (Qijm×n), where Qij is the cross-temporal correlation of the input and A the matrix of Green's (electrotonic transfer) functions of the neuron. That is, if a unit charge is injected at synapse j, Aij is the voltage change recorded i seconds later at synapse i.

If Q has a principal eigenvector then the weights tune to it. This is qualitatively similar to the isopotential situation, but with the correlation matrix smeared and skewed in both space and time. But because Q is asymmetric, it need not have a principal eigenvector. In that case, Q must perform a rotation of the 2D principal eigenspace, and the weights tune to this space and rotate within it.

The theory can be applied to a cable in order to derive a characteristic length scale that is in contradistinction to the input, thereby predicting a relationship between lamination scale and the dendritic length constant of the input's temporal frequency. Supported by ONR and DARPA.

567.20

QUANTAL ANALYSIS IN THE CA3 REGION OF THE HIPPOCAMPUS BY MAXIMUM LIKELIHOOD ESTIMATION. S. Smerin*, R.B. Landgraf, D. Herzen, G. Barretusen, and T.R. Clay, Departments of Behavioral Neuroscience and Biology, University of Pittsburgh, Pittsburgh, PA 15260.

Quantal analysis by the method of maximum likelihood estimation was applied to the mossy fiber synapse and the fimbrial synapse, both of which are on the CA3 pyramidal neuron of the hippocampus. The hippocampus of the rat was sliced and maintained in vitro. Stimulating electrodes were placed in the fimbria and in the s. granuleum of the dentate gyrus. Two hundred to 500 EPSCs evoked from each stimulation site were collected in whole-cell voltage clamping configuration. The probability of observing the peak EPSC amplitudes was formulated in a likelihood function under the assumptions that the probability of release (p) of the quantum follows a simple binomial distribution and the amplitude (a) of the quantum is normally distributed. We obtained the values of p and a by maximizing the likelihood function using an algorithm on the Pittsburgh Supercomputer Center's Cray-YMP.

At the mossy fiber synapse we found that p = 0.7-0.8 and a = 9-12 pA, and at the fimbrial synapse p = 0.3-0.5 and a = 9-10 pA. In this initial study all estimations assumed that the number of release sites was ten. Higher numbers of release sites (up to 600 for the mossy fiber synapse, and up to 10,000 for the fimbrial synapse) will be assumed for future estimations. Quantal analysis by maximum likelihood estimation should be applied before and during the induction of LTP at these synapses. Supported by NS24288.

567.22


The hippocampal mossy-fiber (mf) synapse is attractive for neurophysiological and optical studies of plasticity because of its large size (Yu and Brown, Soc. Neurosci. Abstr. 18, in press; Jaffe and Brown, Soc. Neurosci. Abstr. 18, in press) and electrotonicity proximal to the soma (Siegel et al, Soc. Neurosci. Abstr. 18, in press). The complex circuitry of the CA3 region of the hippocampus, however, makes it difficult to excite the mf synapses selectively when using extracellular microstimulation.

We have devised a set of procedures and criteria for isolating and identifying mf excitatory postsynaptic currents (EPSCs) (Xiang et al., Soc. Neurosci. Abstr. 17, 1991; Claborn et al., submitted). Here we report results of whole-cell recordings of mf EPSCs from rat hippocampal slices that satisfy these criteria and procedures. Slices were cut on a vibratome (450 mm thick) in the plane of the mf projection system (Yu and Brown, ibid). An attempt was made to activate unitary mf inputs by delivering small stimulating currents through optimally positioned 25 mm bipolar stimulating electrodes. The patch pipette access resistance was less than 15 MΩ.

The mf peak conductance averaged 1.2 - 4.7 nS and the net charge transfer from a 80 nV holding potential averaged 1.3 - 3.4 pC. For quantal analysis (Greenwood et al., Soc. Neurosci. Abstr. 18, in press) we explored several response measures, including the peak current, slope of the rising phase, total charge, and partial charge. The fit of quantal models and the estimated quantal parameters depended on the response measure. Supported by NIH and ONR.

567.24


Tetanic stimulation of afferents to area CA1 can induce heterosynaptic PTD. Tetanic stimulation of one input pathway (25 Hz, 15 sec) reduced EPSPs evoked by test stimulation of a second input pathway 60-80%, lasting 4-5 min, when both input pathways converged on the same dendritic domain (apical or basilar dendrites). When inputs did not converge on the same dendritic domain, PTD averaged only 20-25%. While patterns of results seen for both K/APMA and NMDA receptor mediated EPSPs. Tetanic stimulation was followed by a post-tetanic hyperpolarization (PTH) of 3 nV lasting 1-2 min. PTD was prevented by 20 μM DNQX, 50 μM AP5, 500 μM AP, 500 μM CGP 35348, 1 μM Ca, or internal BAPTA and internal Ce. PHT was blocked by DNQX, external and internal Ce, but not by AP, AP5, CGP 35348, or internal BAPTA. PTD may be presynaptically mediated by limited diffusion of an external messenger.

The neostriatum, a part of the basal ganglia involved in motor control and cognitive function, receives a large glutamatergic input from the cortex. Synaptic plasticity at corticostriate synapses has not been well characterized. Using iontophoresis and field recordings in slice preparations of 3.4 wk old and adult rat neostriatum, we have found that high frequency stimulation of glutamatergic afferents leads to both short- and long-term depression of synaptic transmission. Studies were done using superfused slices (4, 100Hz trains 1 duration, 1/10s) and bursts (5 pulses at 100Hz given 5s for 5s). The burst paradigm was included because cortical neuron firing probably more closely resembles bursting than sustained trains. Field potential studies showed that following the train paradigm there was a decrease to 45±16.2% (n=25) of control in the population spike (PS) evoked by single stimuli while the burst paradigm showed a decrease to 49.7±13.5% (n=7) of control. The mean time to recovery was 7.4±2.3 min in the train paradigm and 4.4±2.4 min in the burst paradigm. Overall 38% of slices showed depression that lasted >20 min. Patch/slice studies showed a decrease to 55.4±9.5% (n=7) of control in the EPSP following sustained trains and to 68.75±14.72% (n=8) of control after bursts. For all slices/post cells that showed depression the mean recovery was to 80.6±6.9% of control and this occurred within 5-15 min. For cells that recovered fully the mean time to recovery was 2.7±2.5 min. 47% of cells showed depression lasting >10 min. It was also observed that phorbol diacetate (PDAc) inhibits the decrease in the PS after trains. Without PDAc there was a reduction to 53±5.6% of control but with PDAc a reduction to 95±9.5% of control was seen (n=5).


High-frequency stimulation activates post synaptic N-methyl-D-aspartate (NMDA) receptors and calcium, leading to long-term potentiation (LTP) of synaptic transmission. We hypothesized that a) when NMDA receptors are blocked during high-frequency stimulation, homosynaptic LTD would be unmasked and b) this effect is age dependent. We studied Schaffer collateral-CA1 synapses in hippocampal slices from 15, 30 and 60 day old rats. Two stimulating electrodes were placed in Schaffer collateral axons on opposite sides of extracellular recording electrodes in CA1 pyramidal cell and apical dendritic layers. After a stable baseline period, we tetanized one input (50 Hz, 2.5 μm pulses, 6 trains) while the other served as control. After a brief baseline homosynaptic suppression, we observed either LTD or LTP (30 min post-stimulation) of population spike and e.p.p.s in the tetanized input. LTD was observed in 60% of slices from 60 day old rats (13±5%, n=16) and in 65% of slices from 15 day old rats (45±14%, n=23). whereas LTD was seen in only 13%, 6% and 13% in slices from 60, 30 and 15 day old rats, respectively. LTD was blocked by N-methyl-D-aspartate (NMDA)-blockade with 2-aminophosphononic acid (AP5; 25 μM) was added, LTD occurred in 42% of slices (80±3% of baseline; n=19) from 60 day old rats, in only 14% of slices from 30 day old rats, and in 47% of slices from 15 day old rats (27±6%; n=17), whereas the incidence of LTD decreased to 15%, 14% and 29% in slices from 60, 30 and 15 day old rats, respectively. These studies demonstrate: a) that LTD is largely NMDA dependent in 30 and 60 day old but not in 15 day old rats; b) an unmasking of LTD by blockade of NMDA receptors in slices from 60 and 15 day old rats. Developmental differences in the amplitude of LTD, as well as the extent of NMDA versus non-NMDA LTD, may contribute age-dependent differences in both physiological plasticity and seizure susceptibility. (Supported by American Epilepsy Society and Klingenfuss Foundation.)

567.28 LONG-TERM DEPRESSION (LTD) OF SHAFFER-COLLATERAL TRANSMISSION TO CA1 NEURON INDUCED BY REPEATED APPLICATIONS OF GABA A X. D. Yang* & D. S. Faber Neurobiology Lab, SUNY at Buffalo, Buffalo, NY 14214

While repeated strong stimulation of glutamatergic pathway results in LTD, we previously reported that pairing post synaptic inhibition with weak tetanization of the excitatory pathway led instead to LTD (PNAS vol 86:4829-4830, 1991). The present study shows that repeated application of the inhibitory transmitter GABA alone can also induce LTD of Schaffer collateral transmission. Intracellular recordings were obtained from CA1 neurons in hippocampal slices. The Shaffer collateral pathway was stimulated at 200 Hz to produce the test EPSP. The peak amplitudes were recorded (150 ms) of 10 MM GABA (10 at 40 sec) to the vicinity of the post synaptic neuron resulted in a 50% depression of the EPSP that lasted up to 50 minutes. The duration of the depression tended to be shorter when less GABA was applied (0.5, 1, 100 μM) (n=12). The depression was not due to a decreased input resistance of the post synaptic neuron, and it could be reversed by high frequency stimulation, the paradigm typically used to induce LTD. This depression could be induced in the presence of 50 μM of APV (n=8). Furthermore, to induce a depression similar to that induced by GABA. The above results suggest that repeated action of the inhibitory transmitter GABA causes prolonged depression of glutamatergic transmission, which is likely mediated through the activation of GABA receptors. Together with LTD, this inhibition-induced LTD may play an important role in activity-dependent, long-term synaptic plasticity in mammalian brain.


Long-term potentiation (LTP) is an extensively studied form of synaptic plasticity and putative memory mechanism. While constraints on LTD amplitude are often assumed, it is still unclear whether LTD could play a role in either memory processing or regulating dynamic system stability. Associative LTD can be elicited at Schaffer collateral synapses which display a low-frequency input is negatively correlated in time with a separate high-frequency train of bursts. In contrast to LTD, associative LTD is not blocked by antagonists of N-methyl-D-aspartate (NMDA) receptors. However, LTD is induced by phorbol diacetate (CFs) and a selective inhibitor of gluta- mateglutamatergic synaptic turnover. We report here that low frequency Schaffer collateral stimulation (1Hz-5Hz) in area CA1 of in vitro hippocampal slices induces a non-associative homosynaptic LTD (LTD) of extracellular e.p.s, but only at previously potentiated synapses (1000 Hz/sec, 30min before LTD stimulation). Unlike associative LTD, induction of LTD-LTD was blocked by either the metabotropic antagonist AP5 or the NMDA blocker D-2-amino-5-phosphonovalerate (AP5; 10μM). Furthermore, ionotrophic application of NMDA to CA1 apical dendrites was insufficient to prime synapses for LTD. However, induction of a series of short-term potentiations (6x 30Hz/0.3sec) did prime synapses for subsequent LTD. These results suggest that the threshold for the induction of LTD can be lowered by the previous history of synaptic activity. (Supported by NIMH Grant #45752 and the Office of Naval Research)
A POSTSYNAPTIC ACTION OF SODIUM IONS IS REQUIRED FOR THE INDUCTION OF CEREBELLAR LONG-TERM DEPRESSION IN CULTURED CEREBELLAR PURKHEUROPEURGE NEURONS 

D.L. Lin, A. F. Sugihara, and J.A. Cragg

Long-term depression (LTD) of responses to AMPA test pulses in the tissue cultured mouse Purkinje neuron (PN) is induced when somatostatin-quinquepaleate and PN depolarization are given together. Both AMPA and metabotropic receptor activation are necessary for LTD induction in this paradigm: in PNs clamped to -80 mV in TTX saline, LTD may not be induced by depolarization together with pulses of kACPD or (quinquepaleate+CNOX). The AMPA receptor in these PNs does not appear to exert its effect by directly gating Ca influx (Pca/Pna < 0.2 for AMPA, > 5.0 for NMDA). Replacement of external Na during quinquepaleate depolarization with either the imipramine ion NMG or TEA or the permeant ion Li caused a blockade of LTD induction, suggesting that Na influx through the AMPA associated channel is necessary for this process. To determine whether activation of voltage-gated Na channels could substitute for AMPA receptor activation, responses to AMPA pulses were measured in current clamp mode following ACPD/depolarization conjuction in TTX-free saline. LTD was induced 3/16 times in normal medium and 7/16 times in veratridine (2 µM) indicating that while Na influx via voltage-gated channels may suffice to induce LTD infrequently, activation of AMPA receptors is more effective. Na influx might exert its effects through Na/Ca exchange, a process not stimulated by Li. Antisense to a bovine Na/Ca exchanger shows strong immunoreactivity in our cultured PNs. We are currently employing microfluorometric imaging of Na and Ca to evaluate the potential contribution of this process to LTD induction.


Gangliosides are found to be enriched in the synaptic membranes of the mammalian CNS. Among members of gangliosides, monosialogangliosides such as GM1, and disialoganglioside, GD3, are most abundant in the CNS. Treatment with enzyme neuraminidase (NA) was found to increase mono- and di-sialoganglioside contents. To elucidate extent of their influence, two modes of activity changes in CA1 areas of rat hippocampal slices were used, i.e. synapse potentiation by 3 trains of 100 Hz stimuli on Schaffer's collaterals and synaptic suppression by infusing 5 µM kainate for 20 min. While GM1 enhanced synapse potentiation, GD3 and NA did not have obvious effects. NA suppressed induction of GM1 and GD1 only prevented 40%. NA raised up input resistance of CA1 pyramidal cell and reduced depolarization potential. No change of resting membrane potential was found after NA treatment. However, NA prevented change of input resistance during kainate perfusion. (Supported by CMRF34)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992

We are studying the pharmacology of synapses formed between neurons isolated from neonatal rat superior cervical ganglion (SCG) and atrial myocytes in co-cultures maintained for up to 2 weeks to get the definition of the types of chemical channels (VSCCs) involved in transmitter release. We have found multiple types of VSCCs in these neurons including α1a, α1b, α2A, and α2C (see also Maruhashi et al., this vol.). SCG neurons co-cultured with myocytes are able to release multiple transmitters (Matsumoto et al., J.Neurosci., 7:380, 1987). Evoked synaptic activity was monitored by Fura-2 microfluorimetry of spontaneous calcium oscillations in myocytes. Neurons were blocked by whole-cell patch clamp method, holding cells at −50mV and stimulating at 4 or 10 Hz. Stimulation of 51 pairs examined resulted in increased myocyte oscillation frequency or basal [Ca²⁺] (57%), decrease of basal [Ca²⁺] (41%), or inhibited oscillation frequency (31%). Frequency increases are mimicked by noradrenaline (NA) and blocked by phentolamine. Frequency decrease is mimicked by carbachol (CCh) or by NA and blocked by atropine and atenolol, respectively. CCh decreased, and NA increased, basal [Ca²⁺]. In addition, some synapses appear to be purinergic and/or peptidergic. The pharmacology of the VSCCs involved in transmitter release is being studied by application of toxins: α-Conotoxin GVIA (CCh; αM) was locally applied by pressure injection. Excitatory and inhibitory effects of stimulation were largely blocked by CgTxs. These results indicate that α- and β-Txs sensitive VSCCs (N-channels) play an important role in the regulation of release of a variety of transmitters from sympathetic nerves. PTT is supported by a Fogarty International Fellowship.

568.4 DIFFERENTIAL EFFECTS OF NOREPINEPHRINE ON FIELD POTENTIALS IN LAYERS 1A AND 1B OF RAT OLFACTORY CORTEX. M. C.Vanier* and J.M. Brender, California Institute of Technology, Pasadena, CA 91125.

In rat olfactory (piriform) cortex, layer 1a contains afferents from the olfactory bulb while layer 1b contains intrinsic pyramidal cell, associational fibers. We have previously demonstrated (Haselmo and Bower, J. Neurophysiol. 1992) that the cholinergic agonist carbachol, when bath-applied in a brain slice preparation, causes a large suppression of synaptic transmission in layer 1b of rat olfactory (piriform) cortex while having essentially no effect on layer 1a. These effects were shown to be functionally significantly blocked when translated into a computer model of olfactory cortex (Haselmo, Anderson, and Bower, J. Neurophysiol. 1992). In the present study, we examined the effects of bath-applied norepinephrine in a brain slice preparation on extracellular field potentials in layers 1a and 1b of piriform cortex. We found that 25 mM NE decreased field potential height in layer 1b to 35 ± 11.5% of control, while NE increased field potential height in layer 1a to 139.6 ± 15.1% of control. Both effects were reversible after washout of NE. Thus NE has an effect very similar to ACh in layer 1b, but, unlike ACh, NE increases field potential height in layer 1a. Based on previous modeling efforts, these results are consistent with a role for this neurotransmitter as a ‘state switch’ for associative learning in piriform cortex.


The intermediate lobe of the pituitary (IL) receives a monosynaptic DA projection from the arcuate nucleus which synapses onto a single class of post-synaptic cells, the melanotrophs, which bear D2 receptors. DA release from the neurons innervating the IL is modulated by pre-synaptic autoceptors which are inhibited by D2 antagonists. The IL thus contains both pre- and post-synaptic D2 receptors with different physiological functions. Both pre- and post-synaptic D2 receptor function were assessed simultaneously in a single IL by measuring electrically stimulated (ES) DA release using carbon fiber electrochemical electrodes. (to assess autoreceptor status) and simultaneous intracellular recording from the melanotrophs (to assess post-synaptic D2 receptor status). Haloperidol, a D2 antagonist, increased DA release while simultaneously abolishing the post-synaptic response to SS. Sulpiride, another D2 antagonist, increased post-synaptic DA release by 100% at 50nM, however, sulpiride did not block the post-synaptic hyperpolarization due to SS. Dompieredone, (1μM) another D2 antagonist, blocked the post-synaptic response to SS without increasing DA release, S(-)PPP, thought to effect autoreceptors selectively, increased DA release but had no effect on post-synaptic response while R(+)-PPP caused a post-synaptic hyperpolarization but had no effect on DA release. The ability of these agents to distinguish between these two types of D2 receptors suggests that they may represent subtypes of the D2 receptor. Supported by USPHS 0634 and VA Med Research Service.

568.6 ESTROGEN ATTENUATES α2-ADRENERGIC INHIBITION OF NOREPINEPHRINE RELEASE FROM HYPOTHALAMIC SLICES. G. KARKASHAN*, and A.M. Etgen, Dept. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

The purpose of this study was to determine whether norepinephrine (NE) release in the hypothalamus is controlled by α2-adrenergic inhibition and whether this mechanism is regulated by estrogen. Slices were prepared from female Sprague-Dawley rats that were bilaterally ovariectomized and treated with oil (OVX) or 2 μg of estradiol benzoate (EB) 24 and 48 hrs. prior to sacrifice. Slices were preincubated for 45 min. with 1 μM H-NE and washed for 30 min. in the chambers of a superfusion apparatus at a flow rate of 1ml/5min. Slices were stimulated twice for 3 min. with 10 μM KCl (S1 and S2). S1 and S2 were separated by 24 min. The α1 antagonists idazoxan (IDA) or RX821002 (RX) at 10 μM were applied 15 min. prior to S2 and were present until the end of the experiment. Basal H-NE release was not affected by α1 antagonists or hormone pretreatment. KCl-evoked release of 'H-NE was Cal²⁺ dependent. The amount of H-NE released during S1 was 20% greater in slices from EB-treated animals than in slices from OVX animals. When IDA or RX were infused prior to S2 in slices from OVX animals, H-NE release was increased 50-80% relative to S1. In contrast, IDA and RX had little or no influence on H-NE released during S2 in slices from EB-treated animals. Similar effects of estrogen administration were observed in slices from the preoptic area. These results suggest that α2-adrenergic inhibitory mechanisms are active in the hypothalamus of OVX animals and that these mechanisms are attenuated by estrogen.


To study the effect of substance P (SP) on nociceptive transmission in the dorsal horn of the spinal cord, intracellular recordings were made from substantia gelatinosa (SG, lamina II) and lamina IV-V neurons of the adult rat spinal cord slices. Substance P applied by perfusion produced no changes in membrane potential or conductance in 18 of 18 SG neurons but it increased spontaneous EPSPs in amplitude and frequency in 30% of the SG neurons examined. In 50% of lamina IV-V neurons, SP produced a concentration-dependent depolarization associated with a decreased membrane conductance. The depolarization decreased in amplitude with membrane hyperpolarization and was nullified at about −90 mV. The SP-induced depolarization was not affected by tetrodotoxin, while it was blocked by Co²⁺. Repetitive dorsal root stimulation with intensity sufficient to activate C afferent fibers evoked slow EPSPs that were associated with a decreased membrane conductance in 20% of lamina IV-V neurons. The slow EPSPs decreased in amplitude with membrane hyperpolarization and was nullified at about −90 mV. These neurons were also depolarized by SP. The slow EPSPs and SP-induced depolarization were attenuated by SP receptor antagonist spantide. These observations suggest that SP mediates the slow EPSPs at the synapse between primary afferent and lamina IV-V neurons in the SG.


Medial vestibular neurons in rat brain slices show an endogenous pacemaker discharge. Synaptic modulation of this activity was evaluated by comparing recording of spontaneously active neuronal activity, and iontophoretic application and bath application of various neurotransmitter receptor agonists and antagonists. Iontophoretic application of the muscarinic receptor agonist, oxotremorine-M, increased the firing rate of most neurons and decreased the firing rate in a few. The non-selective muscarinic receptor antagonist, atropine (50 μM), decreased the firing rate (10-27%) in most neurons. Application of the muscarinic Mβ receptor antagonist, gallamine (50 μM), initially increased the firing rate (5-60%) followed by a decrease (10-15%). Little effect was observed with the muscarinic M1 receptor agonist pirenzepine (50 μM) in most neurons.

2-Amino-4-phosphonobutyrate (AP4 100 μM), an agent known to block presynaptic neurotransmitter release, decreased firing rate (10-30%). The GABA_A receptor antagonist, bicuculline (50 μM), increased the firing rate (21-60%) whereas GABA decreased it. Application of bicuculline during the presence of AP4 failed to induce excitation.

Conclusion: The endogenous pacemaker activity of medial vestibular neurons is modulated by various neurotransmitters. Even in isolated brain slice preparations, release of transmitters from presynaptic terminals appears to influence spontaneous neuronal activity, since application of antagonists alters firing rate. Supported by the Aaron Diamond Foundation & NS32807.

Because it is known to enhance neurotransmitter release in vitro, it was of interest to determine whether its previously reported electrophysiological effects on CA1 neurons in the hippocampal slice (reduction in spike frequency adaptation, prolonged duration and increased spontaneous firing) represented direct somatic effects and, if so, to assess the possible role of chloride conductance in mediating these responses. Standard intracellular recording techniques were used to study the effects of 10 μM linopride under conditions of synaptic transmission blockade (omega-conotoxin, CNTX) and an altered chloride equilibrium potential (ECl electrodes). The presence of 0.3 μM CNTX had no influence on the effects of linopride recorded with K acetate electrodes. Conversely, the use of KCl electrodes completely blocked the electrophysiological effects of linopride but had no effect on those of muscarine (thought to act through K channel blockade). These results suggest that linopride exerts direct effects on the CA1 hippocampal soma which may be mediated by the block of a chloride conductance.


There are many studies on pharmacology and underlying mechanisms of neurotransmitter action in cell types of CNS, only a few reports investigate the pharmacological sensitivity of local-circuit interneurons. We investigated the effects of acetylcholine (ACh), noradrenaline (NE), 5-hydroxytryptamine (5-HT) and gamma-aminobutyric acid (GABA) on extracellular recorded single cell spontaneous firing activity in the CA1 field of guinea pig hippocampal slices. Three groups of neurons were investigated: nonpyramidal neurons of stratum radiatum-molecule (N), neurons with single spike discharges of stratum pyramidale (PS) and neurons with complex spike discharges (CS) in the same stratum. Effects of ACh and NE were also tested on p sumed interneurons of str. oriens-pyramidale (I). Similarity between N, I and SS units and their difference from CS units may be suggested on the basis of drug action. Activity of CS units was suppressed by NE, 5-HT and GABA, while in half of these units ACh had biphasic (inhibitory, then excitatory) effects. In contrast, N, I and SS units were activated by NE and ACh. Though 5-HT and GABA suppressed the activity in some N and SS units, many of them were activated. Excitatory influence of NE, ACh and 5-HT on N was preserved with the blockade of synaptic transmission (Fig. 1). The data suggest direct excitatory influence of NE-, ACh- and 5-HTergic afferents on nonpyramidal cells and therefore an indirect inhibitory influence on hippocampal pyramidal cells.

AMPHETAMINE-INDUCED INCREASE IN [3H]TCP INTACT CELL BINDING OF PRIMARY CULTURED NEURON. H.Yamamoto1,2, T.Yamamoto1,2, N.Sagi2, K.Yase2, A.Aba3, T.Moroi1 and K.Yoshikawa2,3. 1Dept. of Psychopharmacology, Tokyo Inst. of Psychiatry, Tokyo 156, 2Dept. of Mol. Biol., Tokyo Metropolitan Inst. for Neurosci., Tokyo 183, Japan.

In order to investigate whether 3H TCP binding, which is known to reflect the physiological condition, we conducted intact cell binding studies in primary cultured neuronal cells derived from fetal rat telencephalon. In modified Locke medium (with 95% O2/5% CO2, 25°C), [3H]TCP (40mM binding to the intact cells was saturable, reversible, and displaced by non-competitive NMDA receptor antagonist or α ligand. Displacement studies of [3H]TCP intact cell binding by αPCP ligands (bupropion, deoxycorticosterone) revealed the presence of the potencies of the inhibition with IC50 values of 0.446, 1.669, 7.02, 127 and 1.206 μM, respectively. The [3H]TCP binding of cells cultured with serum was more sensitive to APV than that of the cells cultured without serum. However, the potency of inhibition by α ligand was not influenced by the culture condition (serum or serum-free medium).

Subchronic exposure to amphetamine increased in the amount of [3H]TCP intact cell binding in a dose-dependent manner. The inhibition of [3H]TCP intact cell binding by α ligands was not influenced by amphetamine treatment. These results suggest that amphetamine-induced changes in neuronal cells is involved in a cellular excitability and formation of reverse tolerance. Furthermore, α ligands may modulate the amphetamine-induced changes through NMDA receptor coupled ion channels.

INTERNEURONAL ELECTROTONIC COUPLING IN A SYSTEM MODEL USING WHITE NOISE ANALYSIS: ETHANOL UNCOPPELES NEURONS. P.Pu, N. Wright, B.L. Bardakjian and P.I. Carlen. 1Physiology Neuroscience Unit, The Toronto Hospital, 399 Bloor St. E., and the Addiction Research Foundation, Toronto, Ont., Canada, MST 2S8.

A system model of dentate granule neurons (Pu et al., IEEE Trans Biomed Eng, 36:1,55-64) using white noise analysis is used to study the electrotonic properties of a multi-segment compartmental circuit including interneuronal coupling, and comprises a combination of resistors and capacitors. Analytical expression of the input resistance is written using the Z-transform. The model parameters are estimated using an optimization procedure from in vitro voltage recordings of the white noise current injected into dentate granule neurons of the rat. White noise analysis allows extraction of the linear component (the first Wiener kernel) of the voltage response. The most appropriate fit to the experimental data (input impedance) required interneuronal electrotonic coupling. Also, the membrane capacitance obtained was close to 1 μF/cm². Acute ethanol exposure (50mM) caused a large increase in the junctional resistance resulting in uncoupling of neurons, as has been shown with higher order alcohols in other cellular systems. Supported by the MRC and ABMRF.

SYNAPTIC RESPONSES RECORDERED FROM BIOCYTIN LABELLED NEURONS IN MOUSE CINGULATE CORTEX IN VITRO. M. Kiraly4 and P.L. Magistretti. 1Inst. de Physiologie, Université de Lausanne, CH-1005 Lausanne, Switzerland.

In a submersed slice (300 μm thick) preparation, intracellular recordings were combined with bicynic injection to characterize the membrane properties, synaptic responses and pharmacological effects of 3-week old mouse anterior cingulate cortex neurons. On the basis of their firing pattern in response to depolarizing current pulses, neurons were classified as regular spiking (Okk6/42) and bursting (6/42). The resting membrane potential (-75 mV to -73 mV), the input resistance (51 MΩ vs 56 MΩ), the time constant (≈ 1 ms vs 7 ms), the action potential amplitude (184 mV vs 79 mV) and duration (1.8 ms vs 1.7 ms) of these two populations were quite similar. Bicynic labelling of regular spiking and bursting neurons revealed pyramidal shaped cells located mainly in layer V and, in a few cases, layers II/III. Stimulation of corpus callosum elicited four synaptic potentials in both cell types : (i) early, monosynaptic EPSP (time-to-peak 6.6 ± 2.4 ms), amplitude 8.7 ± 3.7 mV, blocked by the non-NMDA receptor antagonist CNQX 20 μM; (ii) late monosynaptic IPSP (time-to-peak 11.0 ± 6.4 ms; amplitude 7.0 ± 0.8 mV) blocked by the GABAa antagonist bicuculline 20 μM; (iii) late EPSP (time-to-peak 32.2 ± 6.9 ms; amplitude 10.5 ± 5.9 mV) blocked by the NMDA receptor antagonist AP5 50 μM. This late potential could be enhanced by removal of extracellular Mg2+ and by tonic depolarization of the neuronal membrane. (iv) late IPSP (time-to-peak 101 ± 44 ms; amplitude 3.6 ± 0.5 mV) abolished by the GABAa antagonist saxclofen 300 μM and by a reversal potential of +90 mV, suggesting a K+ channel involvement.
SYNAPTIC PATHWAYS described distal gyrus. Lateral (LP) and medial (MP) perforant pathways terminate on distal and medial portions of dentrites of the dentate granule neurons. Several differences between LP and MP have been described previously. We examined the properties of transmission at these synapses using the whole-cell voltage clamp technique. Experiments were performed on granule neurons in the hippocampal slices taken from 15-30 day old Wistar rats. MP and LP were activated selectively by means of stimulating electrodes placed in the molecular layer. A putative glutamate receptor blocker L-AP4 (20 μM) selectively antagonized synaptic transmission in LP (55% inhibition, S.E. = 9.4%, n = 5). With the use of quantal recordings we have shown that its action was presynaptic. Similar presynaptic actions on LP were observed with application of an NMDA blocker D,L-APV (50 μM). However, in MP the effect of D,L-APV was mainly postsynaptic. The results confirm different locations and characteristics of glutamate receptors in the two pathways. Possible roles for these receptors during high-frequency activity are under investigation. Supported by MRC of Canada.

INHIBITION OF NMDA RECEPTOR-MEDIATED SYNAPTIC CURRENTS BY A MU OPIOID AGONIST IN DISINHIBITED DENTATE GRANULE CELLS. C.W. Xie* and D.L. Lewis. Pediatric Neurology, Duke Univ. Med. Ctr., Durham, NC 27710. We have previously reported that a mu opioid agonist, L-AP10, suppressed GABAergic inhibition and thus indirectly enhanced NMDA receptor-mediated responses in hippocampal dentate gyrus. The present study examined the direct effect of PL017 on NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) of granule cells in the absence of GABAA, inhibition. Isolated NMDA EPSCs were evoked by outer molecular layer stimulation and recorded from granule cells using whole cell voltage clamp techniques in the presence of DNX4 (AMPA antagonist, 10 μM) and bicuculline methiodide (GABAA antagonist, 10-50 μM). The current was enhanced by removing magnesium from the perfusion medium, and completely blocked by D-APV. Bath application of PL017 (3-10 μM) significantly reduced the amplitude of isolated NMDA EPSCs by 27-43%. This effect could be reversed by opiate antagonist naloxone (10 μM). These results indicate a direct inhibition of NMDA currents by mu receptor activation in the dentate, which may serve as a protective mechanism to limit the hyperexcitability of NMDA channels during opioid disinhibition.

L-AP4 AND trans-ACPD REDUCE PAIRED-PULSE DEPRESSION RECORDED FROM THE RAT DENTATE GYRUS MOLECULAR LAYER. J.S. Kalisz* and C.W. Coman. Department of Psychology, University of California, Irvine, CA 92697-5717. The glutamate analogue, L-amino-4-phosphonobutyric acid (L-AP4), has been shown to be a ligand at several glutamate binding sites including: glutamate autoreceptors or AP4 receptors, chloride-dependent transport sites, metabotropic receptors, and NMDA receptors. At low concentrations (10μM), L-AP4 activates the glutamate autoreceptor and reduces field potentials recorded from selected pathways. We have recently observed that applications of low concentrations of L-AP4 (10-20μM) also reduce paired-pulse depression recorded from the perforant path, without a reduction in field potential amplitude. The possibility that L-AP4 may be producing this effect by binding to transport sites, the metabotropic receptors, or NMDA receptors was examined. Paired-pulse depression of field potentials (40-800 ms interstimulus intervals) was recorded from the middle third of the dentate granule molecular layer of rat hippocampus in vivo. The transport inhibitors trans-pyrimidyl-2,4-diaminobutyric acid (25μM) and 1-α-methylisocaprate (20μM) did not alter or mimic the effect of L-AP4 on paired-pulse depression. The NMDA antagonist 2-amino-5-phosphonopentanoic acid (30μM) did not block the action of L-AP4. These results suggest that L-AP4 is not acting at the NMDA receptor or glutamate transport site to reduce paired-pulse depression. However, applications of the metabotropic receptor agonist 1-amino-cyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD; 50μM) reduced paired-pulse depression similarly to L-AP4. It has been shown that L-AP4 has a low affinity for the metabotropic receptor and trans-ACPD may also activate AP4 receptors (Trombley and Westbrok. J. Neurosci., in press). One hypothesis consistent with these results is that trans-ACPD and L-AP4 activate the glutamate autoreceptor to reduce paired-pulse depression.

ACUTE AND CHRONIC EFFECTS OF GLUTAMATE RECEPTOR ANTAGONISTS ON EPILEPTIFORM ACTIVITY IN CULTURE. W.J. Kornobish, and E.J. Furshpan*. Neurology Dept., Mass. General Hospital and Dept. of Neurology, Harvard Medical School, Boston, Ma. 02115. Hippocampal neurons grown chronically with blocking agents (e.g. 1μM kynurenic acid and 11.3 μM Mg2+) show intense epileptiform activity when the blockers are removed (1,2). We have previously shown that the inward polyamine, spermine, activates NMDA receptors to produce high-amplitude synaptic potentials. We have examined the effects of spermine on epileptiform activity. In primary hippocampal cultures, spermine (50 μM) increased epileptiform activity. The results suggest that spermine enhances epileptiform activity by increasing the availability of NMDA receptors.
569.7
NMDA and AMPA RECEPTOR COMPONENTS OF EPSCs FROM RAT DENTATE HILAR INTERNEURONS. T.A. Brown* and R. Dingledine.
Dept. Pharmacology, McGill Univ., North-Chapel Hill, NC 27599.

Spontaneous excitatory post synaptic currents (EPSCs) from interneurons located in the hilar of the dentate gyrus of neonatal rat hippocampal slices were isolated with high positive and negative membrane potentials in 1 mM tetrodotoxin and 10 µM bicuculline. Cells were morphologically identified by a biocytin staining protocol. Marked heterogeneity exists in cellular morphology and degree of NMDA and non-NMDA contribution to EPSC kinetics. At -70 mV EPSCs rose within 2 ms and decayed with a time constant of less than 15 ms which was still fit by a single exponential. The NMDA receptor antagonist D-APV (50 µM) had little or no effect on EPSC kinetics or amplitude at -70 mV while CNQX, a non-NMDA receptor antagonist, made the decay phase slower and the amplitude smaller. At 60 mV both EPSC kinetics and amplitudes were observed between neurons. Some neurons had decay phases best fit with 2 exponentials while others were best fit with one. D-APV always completely blocked the EPSC component best fit with the longer decay time constant. Interestingly, in 5 interneurons, 2 tentatively identified as dentate pyramidal basket neurons and 1 as an eplumiform, EPSCs at +60 mV decayed with a single rapid time constant which was unaffected by D-APV. Diversity in NMDA and non-NMDA receptor contribution to EPSCs suggests different roles for subpopulations of those neurons and may help explain differential sensitivity to insult. (Supported by NS17771 and Bristol-Myers Squibb Company)

569.9
DIFFERENTIAL DEPRESSION OF NMDA RECEPTOR-MEDIATED SYNAPTIC RESPONSES BY MK-801 AND CPP, B. Espin* and R. Capell. Dept. of Pharmacology and Therapeutics, McGill Univ., Montreal, Quebec, H3G 1Y8, Canada.

The influence of repetitive synaptic activation on depression of the NMDA receptor-mediated synaptic responses by a non-competitive antagonist, MK-801, and a competitive antagonist, CPP, was studied in the hippocampal slice preparation of the rat. These responses, evoked by stimulation of the stratum radiatum and recorded in the stratum pyramidale of the CA1 region in slices superfused by a brain homogenate medium, consisted of population spikes (PSs) which followed the primary PS. Both, MK-801 (2 to 100 µM) and CPP (0.2 to 10 µM) applied by superfusion, depressed the number and the amplitude of the secondary PSs in a concentration-dependent manner. The primary PS remained unaffected. In the absence of a drug, repetitive stimulation at 0.2 Hz for 5 min was without any consistent lasting influence on the PS burst. Such stimulation also failed to alter a partially diminished burst during superfusion with the competitive antagonist CPP. In contrast, during superfusion with MK-801, the secondary PSs were greatly diminished immediately after stimulation and in the superfusion medium even with low concentrations of the drug, which produced no or only minimal depression without stimulation. The responses depressed by CPP fully recovered within 30 min, whereas those depressed by MK-801 did not, even after 60 min of superfusion with the drug-free medium. These results demonstrate that, similarly to responses evoked by the exogenous NMDA receptor agonist in cultured cells (MacDonald and Nowak, TIPS 11:167, 1990), the NMDA receptor-mediated synaptic responses evoked by the endogenous neurotransmitter in the slice preparation are depressed by MK-801 in a use-dependent manner. (Supported by the MRC of Canada.)

569.11
INWARDLY RECTIFYING SYNAPTIC CURRENTS ON HIPPOCAMPAL INTERNEURONES. C.J. McCall and D. Dingledine, Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, NC 27599-7305.

Spontaneous miniature EPSCs (mEPSCs) and kainate-evoked currents were recorded from interneurons of CA3 st. radiatum of neonate rat hippocampal slices in the presence of TTX (1 µM) and bicuculline (50 µM). Two distinct populations of interneurons (Type I and Type II) were identified. The I-V relation of kainate in Type I interneurons was linear (Erev = -0 mV). The kainate-I-V relation in Type II interneurons was strongly inwardly rectifying with little or no current at potentials above +50 mV. At -70mV mEPSCs received by Type I interneurons had fast rise times (~1 ms) and decay time constants (~5ms) and were mediated by AMPA receptors. mEPSCs on Type II interneurons had a delayed polarity (~0 mV) and the kinetics of the mEPSCs on Type I interneurons were slow and were comprised of both AMPA and NMDA receptor mediated components. The kinetics of mEPSCs on Type I interneurons were correlated with their rise times and decay times at -70mV. mEPSCs received by Type II interneurons showed extreme inward rectification with no interneurons possessing fast events at +50mV. In a few cells slowly rising currents were recorded following mEPSCs were observed at -50mV. These events were abolished by D-APV and were therefore mediated solely by NMDA receptor activation. On both Type I and II interneurons the rise times of individual mEPSCs were correlated with their decay times and decay time constant, suggesting that the shape of the mEPSC is in part determined by the dendritic origin of the synaptic input. The inward or outward rectification observed in the two interneuron types is likely to be due to expression of different glutamate receptor subunits. Supported by NS17771 and Bristol-Myers Squibb.

569.8

Accumulated evidence suggests a role for redox modulation of NMDA currents in a variety of neuronal preparations. The present study shows that the sulphydryl redox agents dithiothreitol (DTT) and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) differentially antagonize each other's effects on synaptic transmission in rat hippocampus. To reveal the NMDA-mediated component of the synaptic responses, the field potentials evoked by single stimuli were compared to those generated by the fifth of 5 stimuli at 10 Hz. All experiments were performed in saline containing 5 mM K+ to increase the magnitude of NMDA-mediated responses. The conditioned response was 62.5 ± 7.4% (mean ± S.E.M.) larger than the response to a single stimulus (n=12). The NMDA antagonist D,L-2-amino-5-phosphonovarlic acid (250 µM) blocked the potentiation induced by conditioning stimuli. DTT (1 mM) and DTNB (1 mM) were repeatedly applied in the bath. DTT reversibly potentiated the NMDA component of the synaptic potentials (79.7 ± 7.0%, n=17) while DTNB had the opposite effect (408 ± 3.8%, n=17). DTNB did not reverse the potentiation induced by DTT in slices exposed to 300 µM N-ethylmaleimide, an alkylating agent (n=7).

569.10
A NON-GENOMIC ACTION OF ESTROGEN ON SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. M. Wong* and R.I. Mos
dep. Physiolology, UT Southwestern Medical Center, Dallas, TX 75323.

Steroids exert rapid non-genomic effects that are independent of the classical genomic mechanism mediated by intracellular steroid receptors and most likely involve direct interactions with specific membrane receptors. Estrogens can induce a short-term potentiation of the extracellular Ca2+ field potential in the hippocampus (Teyler et al., 1980, Landgren 1992) and of responses to exogenous glutamate in the cerebellum (Smith et al., 1988). The present study investigated the rapid effects of estrogen on responses of individual CA1 neurons to synaptic stimulation and local application of glutamate agonists. Intracellular recordings were made from CA1 neurons in rat hippocampal slices. Synaptic responses were elicited by Schaffer collateral stimulation with a metal electrode and glutamate agonists were applied to the CA1 neurons through an iontophoretic electrode.

Supersuperfusion of 10 Œ M 17B-estradiol, but not 17α-estradiol, significantly increased the amplitude of the Schaffer collateral-induced EPSP, causing a previously subthreshold EPSP to reach threshold. This facilitation of the EPSP by 17B-estradiol usually occurred within minutes and was reversible shortly after washout, suggesting a direct membrane action. The synaptic facilitation still occurred in the presence of the NMDA antagonist, AP5, but was blocked by the non-NMDA antagonist, CNQX. 17B-estradiol also potentiated depolarizing responses to iontophoretic pulses of glutamate and non-NMDA agonists, but not to NMDA. These results suggest that estrogen can induce a rapid potentiation of excitatory synaptic transmission in the hippocampus that involves postsynaptic non-NMDA receptors. (MH41481)

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THURSDAY PM
569.13
ISOFLURANE DEPRIVES A GABA, INHIBITORY PATHWAY IN ISOLATED NEONATAL RAT SPINAL CORD. L. M. Gibbes* and J. S. Kendig. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5123
Volatile general anesthetic agents have been suggested to depress glutamate excitation and enhance GABA inhibition. In this study, volatile anesthetics (0.2-1.2 vol%) were applied in the gas phase to the isolated spinal cords of 1-6 day old Sprague-Dawley rat pups (adult rat anesthetic pressure = 1.4 vol%). The dorsal root potential (DRP) is a population-evoked response which reflects GABA-mediated depolarization of primary afferent nerve terminals. DRP's were recorded from a lumbar dorsal root in response to stimulation of an adjacent dorsal root. Isoflurane reversibly depressed the DRP at all partial pressures (1.2, 0.2 vol%) and were also sensitive to both APV (10 μM) and CNQX (10 μM), suggesting that the GABA-ergic interneurons which mediate the DRP are activated by glutamate acting on both NMDA and non-NMDA receptors. We have previously shown that isoflurane (0.2-1.2 vol%) depresses both NMDA and non-NMDA receptor-mediated ventral root potentials in this preparation. Isoflurane may act directly on GABA receptors to increase inhibition. However, in the circuit which generates the DRP, isoflurane is not on glutamate excitation of the inhibitory interneurons, and inhibition is depressed. Isoflurane thus differs from anesthetic barbiturates, which enhance the DRP.

569.15
MULTIPLE FACTORS MODULATE THE GABA_A IPSP IN GUINEA PIG VENTRAL TEGMENTAL AREA IN VITRO. D.J.Cameron* and J.T.Williams. Vellum Institute, Oregon Health Sciences University, Portland OR 97201.
The ventral tegmental area (VTA) has been implicated by a number of studies in the mediation of addictive behavior. Intracellular recordings were made from VTA cells in a blowfly saline preparation. The majority of cells (35/52) were hyperpolarized by dopamine (100μM) while a smaller proportion (14/50) were hyperpolarized by [met]-enkephalin (ME, 10μM). Approximately half (13/24) of the cells tested were depolarized by 5-HT (30μM). When bipolar electrical stimulation was applied to the preparation, most (29/36) cells exhibited a slow IPSP that was blocked by the GABA_A receptor antagonist, 2-hydroxyasaclofen (10μM). The magnitude of this IPSP was reduced by ME, the κ-agonist U69593 and 5-HT. While naloxone (1μM) reversed the actions of the opioids, the action of 5-HT was not reversed by either the 5-HT_1A receptor antagonist NAN-190 (10mM) or the 5-HT_1B receptor antagonist cyanoindolol (100nM). These results indicate that GABA mediated synaptic potentials in the VTA can be modulated through both μ and κ opioid receptors and by 5-HT, possibly via 5-HT_1B receptors. These findings have important ramifications for understanding the actions of drugs such as morphine and cocaine in the VTA. Supported by an NH&MRC CJ.Martin Fellowship to D.L.C. and by NIH grants DA04523 and MH45003 to J.T.W.

569.16
The LPBN is the recipient of a diverse array of autonomic inputs from more caudal levels of the brainstem. Our prior data demonstrated that both N-methyl-D-aspartate (NMDA) and non-NMDA receptors mediate evoked fast excitatory post-synaptic potentials in vitro (Neurosci Abs 17: 246, 1991). In addition, we have recorded sIPSPs from LPBN neurons and this study examined the role of gamma-aminobutyric acid (GABA) receptors in mediating inhibitory synaptic transmission within this pontine nucleus. LPBN neurons were recorded from coronal (400 μm) slices using whole-cell patch recording. sIPSPs were evoked using platinum bipolar electrodes (10-70V @ 0.2 Hz) placed at the dorsomedial pole of the LPBN. Exogenous application of GABA (1 mM), the selective GABA_A receptor agonist muscimol (1-10μM) and the GABA_A agonist baclofen (10μM) all produced a membrane hyperpolarization (and outward current) that was accompanied by a decrease in the input resistance. Stimulus-evoked sIPSPs or slow inhibitory post-synaptic currents (sIPSCs) reversed at or about the reversal potential of chloride (E_cl). sIPSPs or sIPSCs were attenuated by GABA_A receptor antagonists bicuculline (10-30μM) or picotoxin (100μM) but were unaffected by GABA_B antagonists saclofen or CGP-353684.
These results suggest that evoked sIPSPs and sIPSCs observed in the LPBN are mediated by an increase in membrane conductance to chloride ions and support a role for GABA_A receptors in mediating inhibitory neurotransmission within this nucleus. Supported by Medical Research Council of Canada.

569.17
CELLULAR EFFECTS OF CHOLINERGIC INPUT IN SEPTO-HIPPOCAMPAL SLICES. R. Bianchi* and R.S. Wong**. 1st Dept. of Pharmacology, SUNY-HSC, Brooklyn, NY 11203 and 2nd Dept. of Physiology and Biochemistry, Pisa (Italy) 56100.
In the mammalian CNS the Medial Septum/Diagonal Band complex provides a strong cholinergic projection to the Hippocampal Formation (HF). It has been reported that the CA3 and CA1 regions of the HF slices acutely isolated from adult guinea pigs, in order to study the cholinergic input to the CA3 hippocampal pyramidal cells (HPCs). In such preparation the effects of hexamethonium and atropine on the input resistance and firing in the CA3 HPCs were studied. Intracellular recording from CA3 HPCs showed that these cells can be activated and increase in input resistance and firing in the CA3 HPCs. These effects were mimicked by "bouts" of application of carbachol (CCh), increased by bath application of carbachol (CCh), and bath application of atropine (Atr). Addition of excitatory amino acid blockers (CNQX and CPP 10-20 μM) did not affect the above described effects. We also observed that electrical tetanic stimulation of the septal area or fimbria and CCA application elicited phasic depolarizations when the cells were held more hyperpolarized than -70 mV. Such depolarizations were associated with a decrease of input resistance and blocked by atropine (1μM).

569.18
FTX is a potent blocker of presynaptic Ca++ currents and transmitter release at the mammalian neuromuscular junction (Uchitel et al PNAS 1992). We further studied synaptic transmission in a curarized nerve muscle preparation in order to gain insight into the effects of Cd++ and FTX on transmitter release at low and high frequency nerve stimulation. As expected quantal content was diminished by both agents. In the absence of the blockers high frequency stimulation (40Hz) produces a decline in endplate potential amplitude...

The marine worm toxin anabaseine (2-(3-pyridyl)-3,4,5,6-tetrahydropyridine) structurally resembles nicotine but is even more potent at the frog neuromuscular junction. Previous ligand binding experiments with rat brain synaptosomes demonstrated that anabaseine and the 4-dimethylaminoxylyl (DMAB) adduct interfere with (H)methylcarbamyl choline binding at 100-200 nM concentrations. In contrast with muscle nicotinic receptors, neuronal receptors (rat αβ2 subtype expressed on Xenopus oocytes and PC12 cell subtype expressed in a transfected physiological response to anabaseine relative to ACh (αβ2) or nicotine (PC12)). On the αβ2 subtype anabaseine acted only as a partial agonist (EC50 = 30 μM, relative to ACh EC50 = 2 μM); the peak current was less than half of the ACh maximum inward current. On PC12 cells, anabaseine acted as a full agonist on αβ+ efflux, but was approximately 5x less potent. In contrast, DMAB-anabaseine acted primarily as a nicotinic antagonist on αβ2 and PC12 neuronal nicotinic receptors as well as upon neuromuscular receptors. However, at 100 μM, DMAB-anabaseine produced a small inward current (2% of the maximal ACh-induced current); thus it may possess an extremely small partial agonist activity, at least on the αβ+ subtype. (Partially supported by Taiho Pharmaceutical Co., Ltd.)

KINETIC AND EQUILIBRIUM CHARACTERIZATION OF HIGH-APTIVITY ANALOGS OF VESAMICOL, G.A. Rogers*, W.D. Erecinska, K. Hand, E.M. Parratt, Dept. of Chemistry, Univ. of California, Santa Barbara, CA 93106.

Twelve derivatives of vesamicol have been synthesized that exhibit from three to one thousand times higher affinity for the vesamicol receptor (VR) in Torpedo synaptic vesicles. Kinetic data for the dissociation of analogs from the VR were obtained by a 'base titration' assay (Rogers and Parsons, Neuroreport 1, 22-25, 1990). Equilibrium data were obtained by determining IC50 for [3H]vesamicol from synaptic vesicles at very low concentrations (0.2 μg protein/ml) and for long incubation periods (24 h). Three analogs (2a, 3a and the individual enantiomers) were characterized. The VR exhibited an enantiomeric selectivity ratio of 250 for (S)-4-fluoromethylvesamicol, 4-aminobenzovesamicol (ABV), was radiolabelled with tritium which allowed a direct determination of the equilibrium binding isotherm as well as rates of association and dissociation. (-)-ABV binds to the VR with a Kd value of 6.3 ± 0.5 nM. The rate constants for association and dissociation are (1.0 ± 0.1) x 106 M-1min-1 and (8.4 ± 0.05) x 104 M-1min-1 (t1/2 = 14 hr), respectively. ABV represents a valuable new tool for studies of the cholinergic presynapse.

ION CHANNELS: CELL FUNCTION

MEMBRANE PROPERTIES OF HUMAN FETAL BRAINSTEM NEURONS IN VITRO S. Chung, P.J. Kontu, L.L. Kaczmarek, R. Robbins, B.S. Bunney*. Departments of Pharmacology and Psychiatry, and Neuroendocrinology, Yale Univ. Sch. of Med., New Haven, CT 06510

The whole-cell patch clamp method was used to characterize membrane potentials and currents in human fetal brainstem neurons. Cultures were prepared from fetuses of 9-12 weeks gestation and maintained in culture for 8-13 days prior to recordings. Some cells showed spontaneous membrane depolarizations. In most cells, depolarizing currents triggered short (< 10 ms) action potential that appeared to be mediated by calcium and potassium currents. The majority of inward current was not blocked by TTX. In contrast, 1 mM Ca2+ blocked the current completely indicating that the inward current was probably carried largely by calcium channels. Two major components of outward potassium current were observed. The fast transient component was blocked by 4-AP, but not by TEA. The slow transient component was blocked by TEA but not by 4-AP. In summary, we have identified both potassium and calcium currents in developing human neurons. These cultured brainstem neurons may provide a valuable means to characterize ion channels in human neural membrane.

IONIC MECHANISMS OF OSCILLATORY FIRING ACTIVITY OF RAT CEREBELLAR PURKINJE CELLS. W. Chang*, H. K. Stahlbrandt and C. Strafella. Department of Physiology, Pharmacology and Neuroscience, Texas Tech Health Sciences Center, Lubbock, TX 79430.

Rhythmic firing patterns of neurons have been shown to be mediated by the sequential activation of a set of conductances. However, mechanisms of intrinsic rhythmicity vary considerably among neurons. This study is investigating the contribution of sodium current (INa), inward rectifying cationic current (Ih), transient outward K+ current (Ito) and Ca2+-activated K+ current (IKCa) to oscillatory firing of Purkinje cells. We have confirmed that oscillatory firing activity can be initiated and sustained by membrane ionic conductances in the absence of evidence of action potential-induced release of extrinsic neurotransmitters. Adding TTX to the superfusion solution produced a typical pattern of repetitive burst firing consisting of Ca2+ dependent action potentials (AP) and long periods of hyperpolarization. We used the Ito blocker, cesium, and found that this current probably is not involved with initiation of oscillation, but plays an important role in maintenance of rhythmicity. Cs+ produced long duration hyperpolarization after bursting to very negative membrane potentials (-130 mV) which exceeded the K+ equilibrium potential. Low doses of 4-aminoypyridine (4-AP, Ih blocker) induced oscillatory firing whereas, high doses changed the pattern and frequency of firing, especially the duration and amplitude of action potentials. Apamin (IKCa blocker) reduced part of the afterhyperpolarization duration and after bursting and terminated oscillatory activity. These data indicate an important contribution of Ih, Ito, I4 and Ikca to rhythmic pacemaker firing of Purkinje neurons.

DUAL PATCH-CLAMPING OF MAMMALIAN PURKINJE CELLS IN CEREBELLAR SLICES: M. Sagiport* and R. Linde*, Dept. Physiology/Biophysics, NYU Medical Center, 550 First Avenue, NY, NY 10016.

Double somatic patch recording (P1 and P2) was implemented to study the electrophysiology of Purkinje cells under patch-clamp conditions. One electrode (P1) was utilized to record the neuron and the second (P2) recorded voltage in a current-clamp configuration. The access resistance for electrodes was on the order of 1 mega-ohm and was monitored continuously for P1, by computing the difference in voltage between the two electrodes (P1-P2). Voltage-clamp depolarization steps resulted in either a fully controlled sodium current or, more often, in the generation of action potentials. The latter event occurred most often in cells with less than optimal viability where the leakage resistance was high. The voltage electrode demonstrated action potential generation at the somatic level, even in conditions where the Purkinje cells were properly patched and the access resistance of electrodes was low (1-2 MΩ).

In order to test whether non-somatic sodium spikes could be generated, dendritic depolarization was obtained with glutamate iontophoresis at the distal dendrite. The firing level for the sodium spike in Purkinje cells that ensued was unmodified by voltage-clamp conditions which held the somatic potential at -90 mV and was generated by the initial activation and inactivation of a low-threshold, even in the absence of voltage-dependent calcium conductances. This finding indicates that only one site for sodium spike generation is present in Purkinje cells. The results indicated that sodium spikes and Purkinje cells arise at or near the somatic membrane and that dendrites are unlikely to support sodium spikes, in accordance with previous results. (Supported by NS13742 and AG09480)

INTRINSIC MEMBRANE POTENTIAL OSCILLATIONS IN RAT PRIFORM CORTEX PYRAMIDAL CELLS. B. Burtak* and M.E. Haesting, Dept. Psych., Harvard Univ., Cambridge, MA 02138.

The rat piriform cortex displays oscillatory field potential and EEG dynamics with peaks in the power spectra at 3-10 Hz and 40-60 Hz. These oscillatory properties provide the optimal dynamics for associative memory function in this region (Hasselmo et al., J. Neurophysy. 67:1230-1246). The oscillations have been linked to the time constant of inhibition within the cortex (Wilson and Bower, J. Neurophysy. 67:981-995). However, recent evidence from this laboratory indicates that the oscillitations of action potential-induced release of extrinsic neurotransmitters. Adding TTX to the superfusion solution produced a typical pattern of repetitive burst firing consisting of Ca2+-dependent action potentials and long periods of hyperpolarization. We used the Ito blocker, cesium, and found that this current probably is not involved with initiation of oscillation, but plays an important role in the maintenance of rhythmicity. Cs+ produced long duration hyperpolarization after bursting to very negative membrane potentials (-130 mV) which exceeded the K+ equilibrium potential. Low doses of 4-aminoypyridine (4-AP, Ih blocker) induced oscillatory firing whereas, high doses changed the pattern and frequency of firing, especially the duration and amplitude of action potentials. Apamin (IKCa blocker) reduced part of the afterhyperpolarization duration and after bursting and terminated oscillatory activity. These data indicate an important contribution of Ih, Ito, I4 and Ikca to rhythmic pacemaker firing of Purkinje neurons.

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THURSDAY PM
TONEIC ACTIVATION OF Ig IN CAI PYRAMIDAL NEURONS
Maccagno, G., Janigro, D., Mangoni, M., Zanetti, A., Costa, L.G.

** ** em Environmental Health, Univ. of Washington, Seattle, WA & Dip. di Fisiologia e Biochimica Generali, Milano, Italy

The whole cell variation of the patch clamp technique was used to investigate the role of the hyperpolarization activated inward current (Ih) in CAI pyramidal cells from hippocampal slices maintained in vitro. Under voltage clamp, Ih was activated at potentials between -50 and -150 mV and displayed no time-dependent decay. Manipulations of extracellular, respiratory, and gastrointestinal systems. These neurons are also heterogeneous with regard to several ion conductances. Experiments were thus conducted to determine whether nodose neurons innervating the lung show any distinct electrophysiological features, relative to neurons that were sampled without regard to end organ innervation. Rhodamine or fast blue fluorescent dye was instilled into the airways via an endotracheal catheter in anesthetized guinea pigs. After 5-9 days nodose neurons were acutely dissociated. From 2-5% of the dissociated neurons were retrogradely labeled, and thus taken to be lung-specific. Single microelectrode current- and voltage-clamp techniques revealed physiologically acceptable passive and active membrane parameters in dye-labeled neurons. Lung-specific C-fiber neurons as a population did not differ from non-labeled neurons with respect to several electrophysiological and pharmacological parameters. The most notable exception was a concentration-dependent broadening of the action potential duration in 24/26 lung-specific C-fiber neurons in response to either charybdotoxin or iberiotoxin (1-100 nM). In contrast, the action potential in only 12/23 non-labeled neurons was affected by these peptide toxins. Thus lung-specific C-fiber afferent neurons may be enriched in the expression of a CaV-activated K* channel that is known to be blocked selectively by these peptide toxins.

LUNG SPECIFIC VISUAL C-FIBER NEURONS IN THE GUINEA PIG NODOSE GANGLION EXPRESS A CHARYBDOTOXIN-SENSITIVE K* CONDUCTANCE THAT CONTRIBUTES TO ACTION POTENTIAL DEPOLARIZATION. E.P. Christian & J. Topo, Department of Pharmacology, ICI Americas, Inc., Wilmington, DE 19897.

Afferent neurons in the nodose ganglion innervate organs in the cardiovascular, respiratory, and gastrointestinal systems. These neurons are also heterogeneous with regard to several ion conductances. Experiments were thus conducted to determine whether nodose neurons innervating the lung show any distinct electrophysiological features, relative to neurons that were sampled without regard to end organ innervation. Rhodamine or fast blue fluorescent dye was instilled into the airways via an endotracheal catheter in anesthetized guinea pigs. After 5-9 days nodose neurons were acutely dissociated. From 2-5% of the dissociated neurons were retrogradely labeled, and thus taken to be lung-specific. Single microelectrode current- and voltage-clamp techniques revealed physiologically acceptable passive and active membrane parameters in dye-labeled neurons. Lung-specific C-fiber neurons as a population did not differ from non-labeled neurons with respect to several electrophysiological and pharmacological parameters. The most notable exception was a concentration-dependent broadening of the action potential duration in 24/26 lung-specific C-fiber neurons in response to either charybdotoxin or iberiotoxin (1-100 nM). In contrast, the action potential in only 12/23 non-labeled neurons was affected by these peptide toxins. Thus lung-specific C-fiber afferent neurons may be enriched in the expression of a CaV-activated K* channel that is known to be blocked selectively by these peptide toxins.

REAL-TIME VOLUME MEASUREMENTS IN PRIMARY HIPPOCAMPAL CULTURES BY CONFOCAL MICROSCOPY. M. Repe, M. Fehr, D. Szarowski, D. O. Carpenter and N. Turner. Wadsworth Center for Laboratories and Research, Albany, NY 12201

Activation of non-NMDA channels depolarizes cells by sodium influx and is believed to partially account for cell swelling after anaesthesia or exposure to excitatory agents. Simultaneous measurement of ion fluxes and cell volume may expand the understanding of the cellular regulation of these parameters. Observation of both cell volume and membrane potential can be achieved by simultaneous application of physiologic and microscopic methods. Due to superior depth resolution, confocal microscopy is particularly well suited for real-time imaging of fluorescent labeled live cells. The use of an inverted microscope permits simultaneous real-time recording electrophysiologically while imaging in the confocal mode. Dil is known to label cell membranes with little fading even after repeated exposure to the confocal microscope. Primary hippocampal cultures were incubated for one hour in medium containing 1 μM of Dil and pluron F-127 (0.02%) to facilitate the incorporation of the dye in the cell membrane. Depolarization after application of kainic acid (40 μM) was accompanied by an increase in cell volume after 30 minutes. Cell processes retracted during this time. We conclude that cell swelling after application of kainic acid succeeds depolarization with a time lag of several minutes.

Supported by grants from the Deutsche Forschungsgemeinschaft, NIH RR05904, NS 58910 (KUS and NS 29907).
70.11 ROLEs OF Ca2+ AND K+ IN NERVOUS SYSTEM TUMOR CELL GROWTH. Y. S. Lee, M. Weber, and R. J. Warnecke. Dept. of Physiology, Loyola Univ. of Chicago Medical School, Maywood, IL 60153.

The roles of Ca2+ and K+ fluxes in the tumor cell growth were evaluated. A human neuroblastoma cell line (SK-N-MC) and a human astrocytoma cell line (U-373 MG) were grown in culture with serum-free media containing various ion-channel-related compounds for 1 day culture for cell attachment. The number of cells was measured using a hemocytometer. K+-channel blockers (tetrodotoxin-TEA and 4-aminopyridine-A4P) and Ca2+-channel blockers (verapamil, Ni2+, and La3+) inhibited the growth of both cell lines in a concentration-dependent manner. Both cell lines showed a tremendous decreased cell number when grown in media depleted of free calcium ions by titration with relatively high concentrations (20 mM) of ETGA. High extracellular Ca2+ induced a significant decrease in cell growth. The results suggest that the neuroblastoma cell line were more sensitive to all manipulations used than those of the astrocytoma cell line. Because moderate concentrations of ETGA (0.1-1.0 mM) were ineffective, these cells may have the capacity to continue to proliferate by low Ca2+ levels. Increased intracellular Ca2+ seems to have dual effects: growth-promoting and growth-inhibiting effects. The very steep response-curves of the cells to all the drugs except TEA suggest that these tumor cells have very precise regulatory mechanisms of ion fluxes related to cell proliferation. K+-channel activity and high K+-induced depolarization may influence Ca2+-influx through activation of Ca2+-channels or changes in the electrochemical gradient for Ca2+. Taken together, the results further suggest that although the mechanisms of Ca2+ influx and existence of Ca2+ channels in both cell lines remain to be established, Ca2+-regulating mechanisms may play an important role in tumor cell growth and that ion channels may be potential targets for the management of tumor development.

(Supported by the Mr. and Mrs. Barney Kahn Fund)

70.12 A NOVEL POTASSIUM CURRENT IN ADRENERGIC ZONA FASCICULATA (AZF) CELLS IS INHIBITED BY ADRENOCORTICOTROPIC HORMONE (ACTH) AND ANGIOTENSIN II (AII). B. Milner*, B. A. Rispal, and J. J. Lipsett*. Dept. of Physiology & Pharmacology and Neuroscience Program, The Ohio State University, Columbus, OH 43210-1239.

Adrenal cortical secretion is regulated by peptide hormones including ACTH and AII. The ionic conductances changes associated with peptide-stimulated cortical secretion have not been identified. In whole cell patch clamp recordings from enzymatically dissociated bovine AZF cells, we have identified two separate ionic current components. One is a rapidly inactivating A-type current and a novel rapidly activating, non-inactivating, high unitary conductance channel whose expression is increased by depletion by including 200 μM GTPγS in the pipette. This novel K+ current was potent and completely blocked by AII (100 nM). The mean increase in current was incomplete (mean = 83%). ACTH and AII also depolarized adrenal cells by a maximum of 53 mV and 48 mV respectively with half maximal effects observed at 10 pM and 233 pM. These results indicate that this new K+ channel may set the resting potential of AZF cells.

Further, two different peptide hormones known to work through different second messengers might trigger depolarization-dependent cortical secretion by selective inhibition of this channel.

75.1 EXCITATORY AMINO ACIDS: EXCITOTOXICITY VI


The Ca2+-neurotoxicity hypothesis dictates that neurotoxic phenomena are released when free cytoplasmic Ca2+ ([Ca2+]c) rises too high, for too long. We asked whether depolarization-induced and excitatory-amino acid (EAA)-mediated neuronal injury was due solely to a sustained elevation in [Ca2+]c. Cultured murine spinal neurons were exposed to 50 min challenges with EAA's or high-K+. [Ca2+]c, was imaged with the Ca2+-indicator fura-2. Neuronal death was gauged with trypan blue and ethidium homodimer staining. In 1.6 mM Ca2+, challenges with glutamate (GLU; 250 μM or K+ (50 mM) evoked a transient peak rise in [Ca2+]c, followed by a decay to a lower ‘plateau’, 100% of neurons which died underwent a second, irreversible sustained [Ca2+]c, unaffected by switching perfusions to Ca2+-free solutions indicating that desensitization of neuronal Ca2+-homeostasis precedes a loss of plasma membrane integrity. GLU neurotoxicity was enhanced in the presence of ATP (100 μM), and [Ca2+]c, rapidly [2+]-and peak- and mean increases in [Ca2+]c, but the toxicity of GLU was 5 times greater than that of high-K+. The relationship between Ca2+ influx and neurotoxicity was dissected further by anaerobic treatments. In the absence of free glucose, blockade, GLU (250 μM) and NMDA (100 μM) challenges were lethal within 1.5 hrs of the EAA challenge (85% neuronal death). APV (50 μM) attenuated GLU neurotoxicity by 15% (p<0.001), but NOT the rise in [Ca2+]c, which was only blocked by adding the AMPA/Kainate antagonist CNQX (50 μM). Kainate (KAN; 100 μM) produced a marked rise in [Ca2+]c, which was NOT neurotoxic. The lack of KAIN toxicity was not a consequence of reduced [Ca2+]c, load, because pre-treatment of neurons with Concavanal-A increased KAIN and GLU-induced increases in [Ca2+]c, but had NO effect on neurotoxicity (p=0.34). We conclude that the trigger for Ca2+-neurotoxicity is source-specific, and depends not only on a sustained, elevated Ca2+ load, but on the route of Ca2+ influx.


It is widely believed that glutamate released during conditions such as ischemia kills neurons by increasing intracellular Ca2+ levels. However, determining the specific mechanism responsible for increased [Ca2+]c, particularly during ischemia is not clear. We have previously shown (Soc. Neuro. abstract # 314.14, 1991) that addition of KCN in glucose-free buffer to simulate in vitro chemical ischemia significantly increased the [Ca2+]c, changes induced by glutamate, NMDA, kainate and high K+ in single rat forebrain neurons. We are further studying whether KCN also increases the neurotoxicity of these agonists. Using a dual detection paradigm, monitoring [Ca2+]c, in the presence of Fluo-3, and [K+]c, in the presence of K+-sensitive fura-2, we have been able to simultaneously study the [Ca2+]c, changes and neurotoxicity in cultured neurons. Cells are loaded with fluo-3 (5 μM, 60 min), and recordings are made with propidium iodide (4μM) in the buffer during the experiment. Fluorescence from PI is observed as cells die as this probe is excluded from healthy cells. Almost all the neurons exposed for 5 min. to glutamate 5mM and glycine 1 mM, or kainate show an initial [Ca2+]c, increase and most recover from this Ca2+ load. However, many of those treated with glutamate show a latent rise in [Ca2+]c, with a variable time delay (2-3 hrs). KCN (5 mM) pretreatment appears to enhance the initial [Ca2+]c, rise and also shorten the latency period for the delayed irreversible [Ca2+]c, rise. However, most neurons treated with 100 μM kainate or KCN and kainate did not show a delayed [Ca2+]c, rise within 3.5 hrs of experiment duration. Cell death, measured by an increase in PI fluorescence, usually occurs following the late [Ca2+]c, rise. (Supported by an American Heart Association Grant-In-Aid)

5.1.4 INTRA-NEURONAL Ca2+-BUFFERING WITH BAPTA Enhances GLUTAMATE EXCITOTOXICITY IN VITRO AND ISCHEMIC DAMAGE IN VIVO K.G. Rambidge* and K.M. Abdel-Hamid. Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6Z 1Z3.

Primary cultures of rat hippocampal pyramidal neurons were exposed for 30 min at room temperature to various levels of glutamate. Survival was assessed 48 h later using the fluorescent vital stains, calcium/am and ethidium homodimer. Compared to controls, prior loading of the neurons for 60 min in a 10 μM solution of the fast Ca2+-buffer BAPTA-AM greatly increased the excitotoxic effect of glutamate. The BAPTA-AM loading alone produced no observable toxicity although it was demonstrated using fura-2 that the rate of rise and maximum amplitude of glutamate induced transients [Ca2+]c were reduced whereas their decay times were considerably extended; consistent with the action of an enhanced Ca2+-buffering capacity.
571.5 DIMINISHED GLUTAMATE-INDUCED INFUX OF EXTRACELLULAR CALCIUM IN THE EPILEPTOGENIC HUMAN HIPPOCAMPUTUS

Dennis D. Spencer* and Matthew L. Judson

Section of Neurosurgery and Neuroendocrine Program, Yale University School of Medicine, New Haven, CT 06510.

We have developed and characterized a microdialysis probe suitable for chronic implantation in the human hippocampus. These probes were implanted bilaterally in patients with intractable complex partial epilepsy. To test the hypothesis that clinical, spontaneous-onset seizures emanate exclusively from regions exhibiting extracellular glutamate concentrations and that repeated exposure to these concentrations results in a loss of glutamate receptor-mediated responses, we measured extracellular glutamate levels in spontaneous-onset seizures and the flux in Ca** in rats following the local perfusion of excitogenous glutamate.

During seizures, extracellular glutamate reached concentrations which are potentially neurotoxic (100 μM). Moreover, the increase was greater and sustained in the epileptogenic hippocampus.

Furthermore, glutamate-induced calcium influx was diminished in the epileptogenic hippocampus (39±6% decrease in the non-epileptogenic vs. a 17±7% decrease in the epileptogenic side) consistent with prior excitotoxic injury and suggesting loss of receptors and/or cells which express glutamate-gated calcium channels.

Supported in part by the NIH.

571.7 POST-ISCHEMIC GLUCOCORTICOID EXPOSURE DOES NOT AUGMENT EXCITATORY AMINO ACID OVERTOW IN THE RAT HIPPOCAMPUTUS. G. Tombaugh and R. M. Sapolsky, Dept. of Biol. Science, Stanford, CA 94305.

Exposure to glucocorticoids (GCs) can enhance hippocampal neuron loss following ischemia in rodents. Recent findings from our lab suggest that GCs, when present both before and after ischemic-hypoxic insult, increase the extracellular level excitatory amino acids (EAA's) in the rat hippocampus (J. Neurochem, 1992). This implies that GCs may exacerbate injury by exacerbating excitoxic cascade. In light of this, we asked whether post-ischemic exposure to GCs could influence the extracellular levels of EAA's in the rat hippocampus after transient forebrain ischemia. Rats (n=10) were surgically prepared for 4-vehicle occlusion, adenorectomized, and food-deprived overnight while given access to 0.9% saline. The next day, animals were subjected to 20' of global forebrain ischemia under halothane anesthesia. Rectal temperature was maintained at 37°C with heating lamps and cortical EEG amplitude was monitored throughout the experiment. Immediately after occlusion, half of the rats received injections of corticosterone (CORT) resulting in plasma CORT levels that matched those seen in intact rats following a 20' ischemic insult; control rats received equivalent vehicle injections. Hippocampal microdialysis samples were collected during and for 8 hours after ischemia and then analyzed for selected amino acids by HPLC. A large transient rise in EAA's was seen in both treatment groups during the ischemic period, but no differences between groups were detected at any time point for any amino acid. These results suggest that post-ischemic GC exposure in ADX rats does not endanger hippocampal neurons by elevating interstitial levels of EAA's.

571.9 TRANSNEURAL MECHANISM OF SELECTIVE DEATH OF CA1 NEURONS BY AN NMDA RECEPTOR AGONIST, L-CCG-IV. T. Shigemori, G. Kan, Y. Yamazaki, M. Takeda, and H. Shimozaki

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2Toho Pharmacological, Pharmacology, The Tokyo Metropolitan Institute of Medical Science, Japan.

We have previously reported that intracerebral injection with a potent NMDA receptor agonist, (2S,3S,4S)-N-methyldehydroaspartate in the CA1 of the rat hippocampus. Although the injection site was unilateral CA1, neuronal death frequently occurred in the blastic zone. Therefore, we asked to determine whether or not the severance of corpus callosum would influence the events in the contralateral side. We employed three groups of adult rats under halothane anesthesia, (1) injection of L-CCG-IIV (50 μM) into the unilateral CA1, (2) severance of corpus callosum followed by injection of L-CCG-IIV and (3) injection of a metabolic receptor agonist, the (2S,3S,4S)-N-methyldehydroaspartate in the unilateral CA1. After 1 week, the brain was examined for histopathology. The intact neurons in the CA1 group were counted. As shown below, L-CCG-IIV significantly killed neurons ipsilateral to injection. There was also massive reduction in cell number contralateral to injection, though statistical significance could not be obtained. In contrast, when combined with severance of corpus callosum, there was no neuronal death in the bilateral CA1. L-CCG-IIV did not cause neuronal death either, as we previously reported. We conclude that transneural mechanism of selective death is due to excitotoxicity of CA1 neurons by glutamate excitotoxicity. The intrinsic neuronal circuitry in and around the hippocampus would undergo this phenomenon.

Neuronal Density in the CA1 Sector (cells/mm^3): (a) ipsilateral contralateral L-CCG-IIV 1646 ± 31 pg/mg p0.03 79 ± 7 pg/mg p0.03 Severance of Corpus Callosum & L-CCG-IIV (1) 1646 ± 31 pg/mg p0.03 79 ± 7 pg/mg (2) 1646 ± 31 pg/mg (3) 1646 ± 31 pg/mg (4) 1646 ± 31 pg/mg


Glutamate and excitatory neurotransmitters stimulate calcium influx into neurons and glial cells. Excess calcium accumulation may underlie ischemic brain injury as well. To study kinetics of calcium influx in response to glutamate or ischemia, we measured Ca** uptake and washout from hippocampal brain slices. Ca** kinetics was studied in 500 μm thick control slices, 500 μm slices in the presence of 100 μM glutamate in 1000 μM thick hippocampal slices which serve as our model of the ischemic penumbra.

We have derived three and four compartment kinetic models but report results only for a serial three compartment, four parameter model fit by weighted least squares analysis. Results with this kinetic model show that glutamate increases influx of calcium into the first tissue compartment without affecting efflux from the first compartment or either parameter for the second tissue compartment. Results with ischemic 1000 μm slices show that first compartment constants are the same as control 500 μm slices but that entry into the second compartment is significantly increased. Concentration response curves for the stimulation of calcium influx into 500 μm slices revealed relatively little variation with concentration, with a trend toward highest calcium influx at or below 1 μM.

Our results suggest that calcium accumulation in brain during ischemia occurs as a result of factors not solely related to glutamate excitotoxicity, but also to factors that increase calcium in intracellular compartments which are not affected by glutamate under physiological conditions. Further work will be done to clarify the kinetic parameters, study long term correlations and receptor analogues and extend the method to autoradiography.

571.8 A MASS-FRAGMENTOGRAPHIC SEARCH FOR 6-HYDROXYDOPAMINE (6HO-DA) IN THE RAT BRAIN FOLLOWING THE ADMINISTRATION OF PSYCHOSTIMULANTS: A NEGATIVE FINDING. L. Kegourc and D. J. Wyatt

NBP, NIH, St. Elizabeth Hospital, Washington, D.C. U.S.A.

Non-enzymatic formation of 6-HO-DA from dopamine following the administration of psychostimulants has been hypothesized to mediate their neurodegenerative effects. Direct support for this hypothesis came from the report of a 6-HO-DA-like substance in the rat striatum (ST) following the administration of high doses of amphetamine (Pharmacol. Biochem. Behav. 1984, 21, 29-31). Although the identity of this 6-HO-DA-like substance was established by indirect pharmacological approaches, its structure has not been conclusively confirmed by direct methods. We used a mass-fragmentographic method to search for 6-HO-DA in the rat frontal cortex (FC) and ST following the administration of 6-Hydroxy-dopamine and a number of drugs that stimulate dopamine release. 6-HO-DA identity was based on the presence of specific fragments in its spectra produced by positive and negative chemical ionization. While both p.i. and i.c.v. administrations of 6-Hydroxy-dopamine (2 mg/kg and 200 μg respectively) produced measurable concentrations of 6-HO-DA in the FC and ST, no 6-HO-DA was detected after 25 or 50 mg/kg of methamphetamine, amphetamine or after 20 mg/kg of cocaine. It is concluded that if these agents can cause the formation of 6-HO-DA, the concentrations produced are below the detection level of our assay (less than 50 pg/mg protein).
571.11 NEURODEGENERATION MEDIATED BY PLATELET-ACTIVATING FACTOR (PAF) RECEPTORS
Platelet-Activating Factor (PAF) is a naturally occurring alkyl-either phospholipid which serves as an intercellular messenger in a variety of physiologic and pathologic processes. The presence of PAF antagonists in experimental models has implicated PAF in a wide range of diseases, including brain damage induced by anoxia and ischemia. The ability of PAF to cause an increase in intracellular levels of calcium (Korneci and Ehrlich, Science, 240: 1792, 1988; Linds, 26: 1247, 1991) has suggested that, in analogy to the action of glutamate, PAF may have excitotoxic activity.
In the present study we have examined this possibility directly by testing the effects of PAF on primary cultures of central nervous system neurons. CNS neurons were cultured from the telencephalon of 7-day chick embryos and maintained in vitro for a 4-day period during which they undergo differentiation and maturation. Neurodegeneration was observed microscopically and quantified by measuring release of lactate dehydrogenase (LDH). PAF-induced degeneration of these cultured CNS neurons was found to be both concentration-dependent (1nm to 1µM) and time-dependent (30 min to 96 hrs). At saturation, PAF caused 35% neurodegeneration. Presumably, only those neurons in the heterogeneous population that have PAF receptors are sensitive to its excitotoxic action. This possibility was supported by the finding that two structurally different antagonists of PAF receptors, the thromboxane antagonist WEB2086 and the ginkgo-biloba BNS2021, completely blocked the neurodegenerating effects of PAF. Antagonists of PAF may prove useful in the treatment and/or prevention of neurodegenerative stroke and spinal cord injury. Supported in part by a grant from the American Paralysis Association.

571.12 INTRATHECALLY ADMINISTERED ACROLEIN ACID INDUCES LONG-LASTING SPASTIC PARAPARESIS WITH DAMAGE OF SPINAL NEURONS IN THE RAT.
Kwak S., Abraham H., Nakamura R.
National Institute of Neuroscience, Kodaira, Tokyo 187, Japan.
Since systemic administration of acrolein (ACRO) induces selective damage of spinal interneurons in the rat, direct neurotoxic activity of ACRO was investigated in spinal interneurons in vivo. ACRO, a kainic acid and AMPA were injected intrathecally at a constant rate for 2 hours through a small tube placed in the L5/S6 subarachnoid space of adult rats. ACRO induced an evident spinal behavioral change in a dose-dependent manner at the concentrations above 2 µM; forced extension of hindlimbs, defecation, fascination and tremor of hindlimbs and flaccid paraplegia. At the concentration of 10 µM of ACRO, 30% of the rats developed spastic paraparesis on the next day with degenerating small or medium sized neurons in the caudal spinal segments, as seen in the spastic rats induced by systemic administration of ACRO. This paraparesis remained unchanged for months. Coinjection of 1 ml CNQX but not APV ameliorated the behavioral and neuropathological changes induced by ACRO. Kainic acid also induced similar behavioral and neuropathological changes but is more than 10 times weaker than ACRO. AMPA induced quite different behavioral changes; rats extended their hindlimbs periodically at concentrations above 1 µM with pathological changes in the spinal neurons. This study suggests that ACRO has potent kainate-like, rather than AMPA-like, excitotoxic activity on the spinal neurolesions of the ACRO injection provides a useful tool for investigating neuronal death of spinal neurons and also for the investigation of spastic paraparesis of spinal origin.

571.13 THE EFFECTS OF IBOTENIC ACID LESIONS OF THE VENTRAL Tegmentum ON BRAIN STIMULATION REWARD.
R. Anderson*, M. Trzcinska and E. Milejczyk.
Behavioural Neurophysiology Lab., Univ. of Ottawa, Sch. of Psy., 275 Richmond St., Ottawa, Ontario, K1N 6N5.
Until recently, it was not possible to differentiate between the role of cell bodies and fibers of passage in brain stimulation reward (BSR). If BSR within the ventral tegmental (VT) is due to the activation of cell bodies, an intra-VT injection of ibotenic acid (IBO), a neurotoxin that destroys cell bodies while presumably sparing fibers of passage should then decrease the rewarding efficacy of the stimulation. We obtained the rate-frequency functions (RF) of VT self-stimulation (relating barpressing rate to the frequency of cathodal pulses) for a series of pulse intensities, before and after an intra-VT injection of 4 µg of IBO in rats implanted with a chemiret (a combination of electrode and injection cannula). The injection of IBO shifted the RF functions toward higher frequencies, indicating a decrease in the rewarding stimulus efficacy. Subsequent histology reveals no primary demyelination, the data will point to an important role of VT cell bodies in BSR.

571.15 AMINOXYACETIC ACID (AOAA) POTENTIATES EXCITOTOXIC BRAIN INJURY IN PERINATAL RATS.
J.W. McDonald* and D.D. Schwartz.
Lilly Research Laboratories, E1 Lilly and Company, Indianapolis, Indiana 46225
AOAA, an inhibitor of mitochondrial malate-aspartate shuttling, was assayed for its ability to potentiate excitotoxic brain injury in perinatal rats. Unilateral intrastriatal injection of NMDA (40µg, 2 µL, D-2-O, 3H label) of AOAA in PND 7 rats produced prolonged, dose-dependent tonic-clonic seizures and unilateral brain injury over a 1.4 µmol dose range. Intrastriatal injection of 1 µmol,5 µl AOAA on PND 7 resulted in significant brain injury when assayed on PND 12 by comparison of cerebral hemisphere weight disparities (p<0.05 ANOVA, LSD post hoc, n=6). AMCA treated vs vehicle treated, 0.10±0.5% damage, n=5). Co-intrastriatal injection of 1 µmol AOAA with subtoxic doses of either NMDA, AMPA or 10 µM-ACPD markedly potentiated the severity of behavioral, histologic and neuronal injury (p<0.05). Intracerebral injection of 1 µmol AOAA when assayed by hemisphere weight disparities.

571.16 EFFECT OF SOMAN AND ACHE ON GLUTAMATE-INDUCED NEUROTOXICITY IN CULTURED CEREBRAL CORtical NEURONS.
S.S. Deshpande, R. Ray* and M.G. Fillert.
Neurotoxicology Branch, Pathophysiology Division, USAMRICD, Aberdeen Proving Ground, MD 21010-5429.
Soman (pinacolylmethylphosphonofluoridate), an irreversible inhibitor of acetylcholinesterase, causes seizure-related brain damage in animals. Extensive neuropathology has been observed in the pyriform cortex, hippocampus, amygdala and thalamus of rats and guinea pigs (Churchill et al., J NeuroToxicology 6, 869, 1986; Sparenberg et al., NeuroToxicology 11, 509-520, 1990). To investigate the link between soman-induced neuropathology and glutamate (GLU) excitotoxicity, we used dissociated cell cultures derived from embryonic rats (E17 day) in organotypic culture. In 14 days in culture, the cells were exposed to GLU (50 µM) alone or in combination with ACh (100 nM) and soman (100 nM). Cytotoxicity was assessed by 24 hr by trypan blue exclusion and LDH release in the extracellular medium. Complete inhibition of ACHE by 100 nM soman had no effect on the cell viability. Exposure to a nontoxic concentration of GLU (50 µM) in the presence of 100 nM ACh produced a 3 fold increase in LHD release confirming earlier observations of Matson (Brain Res., 497, 402-406, 1989) that ACh lowers the threshold for GLU-induced neurotoxicity. Exposure to 50 µM GLU plus 100 nM ACh in combination with 100 nM soman contrary to the expectation did not significantly potentiate cytotoxicity further. In conclusion, soman does not exert a direct toxic effect on cerebral cortical neuronal culture. The degeneration-potentiation effect of ACh emphasizes the importance of multiple transmitter inputs involved in neuronal degeneration.
572.1 ISOLATION OF NOVEL METABOTROPIC GLUTAMATE RECEPTOR cDNAs FROM RAT OLFACTORY BULB. L.A. Saugstad, T.P. Segerson, E.E. Muthukumar, and G.L. Westbrook. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences Univ., Portland, OR 97201 and Departments of Molecular and Cellular Biology and DNA Chemistry, Zymogenetics, Seattle, WA 98109.

Glutamate receptors are grouped into two broad classes: the ionotropic receptors which are cation-selective ion channels, and the metabotropic receptors (mGluRs) which are G protein-coupled receptors. Several distinct metabotropic receptor cDNAs have been isolated to date (Mans et al., 1991; Houamed et al., 1991; Tanabe et al., 1991; Conn et al., 1992). mGluR1 has been sequenced as the receptor that inhibits transmitter release at all pathways. This inhibition is blocked by PTX, suggesting a direct interaction via a Gi/Go protein with voltage dependent calcium channels. L-AP4 does not activate mGluR1. Therefore, this receptor appears to be pharmacologically and functionally distinct. In physiological experiments (Trombley and Westbrook, 1992) the AP4-specific receptor(s) appear to be abundant on mitral cells of the olfactory bulb. In order to identify and characterize the AP4-specific receptor, we have amplified rat olfactory bulb cDNA using the polymerase chain reaction and degenerate oligonucleotide primers to the conserved transmembrane domains three and six of mGluR1 and mGlu4. Sequence analysis of the amplification products revealed the presence of at least 10 mGluRs are distinct, but related to the previously isolated metabotropic receptor cDNAs. We are currently screening a rat olfactory bulb cDNA library with the amplification products to isolate full-length cDNA clones. As several of the previously cloned mGluRs show distinct expression patterns in the olfactory bulb, isolation and expression of these related receptors should provide a handle for exploring the function of these receptors in the olfactory system. Supported by the Klingenstein Foundation and NIH T32 DK07680.

572.2 INHIBITION OF cAMP FORMATION BY METABOTROPIC RECEPTOR AGONISTS IN BRAIN SLICES: DEVELOPMENTAL PROFILE AND PHENOMENOLOGICAL CHARACTERIZATION. A.A. Genazzani, G. Casabona, G. Alonso, M. Di Stefano, E. De Bernardis, M.A. Bortone and F. Nicoletti. Institute of Pharmacology, University of Catania, Italy.

1,3-ACPD inhibited forskolin-stimulated cAMP formation in slices from adult rat hippocampus and hypothalamus. Quisqualate and ibotenate were much less efficient than 1,3-ACPD in inhibiting forskolin-stimulated cAMP formation, whereas BMAA, AMPA or NMDA were inactive. Stimulation of cAMP formation by forskolin was attenuated by the enzadine antagonist (A15), which depletes the endogenous adenosine. The inhibitory action of ACPD was no longer visible when forskolin was added to the slices in the presence of ADA. In addition, ACPD failed to inhibit forskolin-stimulated cAMP formation in hippocampal or hypothalamic slices prepared from rats at 1, 8 or 15 days of postnatal life. These results suggest that hippocampal or hypothalamic slices express a metabotropic receptor subtype that is negatively coupled to adenylate cyclase. This is preferentially activated by ACPD, is expressed in the adult life and interacts with endogenous adenosine through a mechanism that remains to be elucidated.

572.3 METABOTROPIC GLUTAMATE RECEPTOR-MEDIATED INCREASES IN CYCLIC AMP ACCUMULATION IN THE HIPPOCAMPUS. P.J. Conn and D.G. Winder. Dept. of Pharmacol. and Program in Neuroscience, Emory Univ., Atlanta, GA 30322.

The most well-characterized metabotropic glutamate receptor (mGluR) subtype is coupled to activation of phosphoinositide hydrolysis. However, little is known about other second messenger systems that are activated by mGluRs. Thus, we performed a series of experiments to test the hypothesis that mGluR activation leads to increases in cAMP accumulation in rat hippocampus. We found that the selective mGluR agonist (1S,3R)-1-amino-cyclopentane-2,3-dicarboxylic acid (1S,3R-ACPD) induces a concentration-dependent increase in cAMP accumulation in cross-hopped hippocampal slices. Furthermore, the 1S,3R-ACPD increase in cAMP accumulation is blocked by the mGluR antagonist L-serine-O-phosphate (L-SOP), L-2-amino-3-phosphonopropionic acid (L-AP3), L-2-amino-4-phosphonobutyric acid (L-AP4), but not by selective antagonists at ionotropic glutamate receptors. Taken together, these data suggest that 1S,3R-ACPD-stimulated increases in cAMP accumulation are mediated by activation of mGluRs.

572.5 THE METABOTROPIC GLUTAMATE RECEPTOR IS COUPLED TO ADENYLATE CYCLASE IN PRIMARY CULTURES FROM MOUSE CEREBRAL CORTEX. E. Rami, P. Micheli, P. Ferragili, F. T. M. van Amsterdam, G. Gabrielli, C. Cavicchioli and E. Negrini. (1) Glasso Research Laboratories, Verona, Italy; (2) Institute of Pharmacology, University of Catania, Italy.

Recent evidence has suggested the existence of multiple subtypes of metabotropic glutamate receptors (Tanabe et al, Neuron, 8, 1992). Whereas the mGluR1 subtype is coupled to phosphoinositide (PPi) hydrolysis, the mGluR2 subtype is negatively linked to adenylate cyclase. In situ hybridization demonstrated that mGluR1 and mGlu2 receptors differ in their anatomical distribution, with selective innervation of the dentate gyrus. We now report the presence of a metabotropic receptor negatively coupled to adenylate cyclase in primary cultures from mouse cerebral cortex. Cultures at 14 days of maturation in vitro were stimulated with forskolin or isoproterenol, which both increase cAMP formation. This stimulation of the metabotropic receptor agonist, quisqualate and 1S,3R-ACPD, which also reduced basal cAMP levels. These results support the existence of a metabotropic receptor subtype coupled to adenylate cyclase in cortical cells.

572.6 TRANS-1-AMINOCYCLOPENTANE-1,3-DICARBOXYLIC ACID-INDUCED PHOSPHOINOSITIDE HYDROLYSIS AND MODULATION OF NEURONAL EXCITABILITY DO NOT UNDERGO PARALLEL DEVELOPMENTAL REGULATION. V. Bose, M.A. Desai, T.S. Smith and P.J. Conn. Pharmacology Dept., Emory University, Atlanta, GA.

The selective metabotropic glutamate receptor agonist, trans-1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD), stimulates phosphoinositide hydrolysis and elicits a number of electrophysiological responses in hippocampus. If these effects are mediated by the same receptor, then they should undergo parallel developmental regulation. Therefore, we compared the phosphoinositide hydrolysis response and the electrophysiological responses to trans-ACPD at two different developmental stages. Trans-ACPD-stimulated phosphoinositide hydrolysis was much greater in hippocampal slices from immature (6-11d old) rats than from adults. In contrast, trans-ACPD elicited decreases in spike frequency adaptation and in the amplitude of the slow afterhyperpolarization in roughly equal percentages of immature and adult CA1 pyramidal cells. Similar results were obtained using the putative endogenous agonist, glutamate. These data support the hypothesis that certain electrophysiological effects of trans-ACPD are mediated by a metabotropic glutamate receptor that is distinct from the phosphoinositide hydrolysis-linked glutamate receptor.

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572.7 MODULATION OF NMDA RECEPTOR-MEDIATED TRANSMISSION IN CEREBRAL GRANULE CELLS BY METABOTROPIC GLUTAMATE RECEPTORS AND NITRIC OXIDE. D.J. Rossi*, G.A. Kinney and N.T. Slater. Dept. of Physiology, Northwestern Univ. Med. School, 303 E. Chicago Ave, Chicago, IL 60611. Excitatory amino acid transmission in rat granule cell (GC) synapse in cerebellum is mediated by both AMPA and NMDA subtypes of glutamate receptor. The activation of the receptors is mediated by the release of a calcium wave, which is thought to play a crucial role in regulating synaptic efficiency. We have examined the effects of metabotropic glutamate receptor (mGluR) agonists and the NO donor nitroprusside (SNP) on both AMPA and NMDA receptors in guinea pig neuroblastoma-astrocytoma (NG108-15) cells. We observed that SNP, a NO donor, produced a significant increase in the synaptic transmission, while the mGluR agonist (2S,3R)-ACPD produced a significant decrease. The results suggest a role for NO in modulating the glutamatergic transmission in the cerebellum.

572.8 ACTIVATION OF METABOTROPIC EXCITATORY AMINO ACID RECEPTOR POTENTIATES AMPA AND NMDA RESPONSES OF SPINAL DORSAL HORN NEURONS. R. Carrier*, L. Fusin and M. Randy. Department of Veterinary Physiology and Pharmacology, Iowa State University, Ames, IA 50011. In freshly isolated spinal dorsal horn neurones (lamina I-V) of the young rat, the effects of activation of metabotropic glutamate receptors were studied. The activation of metabotropic glutamate receptors (mGluR) produced a significant potentiation of AMPA and NMDA responses, which were observed upon the application of (3S,3′R)-ACPD. The potentiation was observed in a dose-dependent manner and was maximal at 100 nM. The results suggest a role for metabotropic glutamate receptors in modulating the excitatory amino acid transmission in the spinal cord.

572.9 ACTIVATION OF METABOTROPIC RECEPTORS DIFFERENTIALLY MODULATES EXCITATORY AND INHIBITORY SYNAPTIC TRANSMISSION BETWEEN RAT HIPPOCAMPAL NEURONS IN CULTURE. M. B. Ghatarelli*, A. R. Wilcox and M. A. Dichter. Dept. of Pharmacology, Physiology and Neurology, Univ. of Pennsylvania School of Medicine and Graduate Hospital, Philadelphia, PA, 19134. Glutamate receptors can be classified into ionotropic and metabotropic receptors, the latter possibly mediating their effects through an increase in membrane phosphatidyl-inositol hydrolysis. We have studied the effects of metabotropic receptor activation on synaptic transmission in hippocampal cultures using the whole-cell voltage-clamp technique. Activation of mGluR (100 μM) in the presence of appropriate ionotropic receptor antagonist reversibly increased the AMPA/NMDA ratio in the hippocampal slices. The ratio was also increased in primary synaptic transmission in hippocampal cultures using the whole-cell voltage-clamp technique.

572.10 METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION ERODES THE RELEASE OF ENDOPHASIC GABA FROM RAT CORTICAL SYNAPTOSOMES. L. Diaz-Arnedo*, R. K. Kendall and P.C. Emsen* MRC Group and Department of Neurobiology, University of Glasgow, Glasgow, Scotland, U.K. Metabotropic glutamate receptor (mGluR) mRNA is expressed in many cells of the mammalian central nervous system, including astrocytes. In young rat brain, the mGluR-mediated activation of synaptic transmission in hippocampal cultures using the whole-cell voltage-clamp technique.

572.11 POTENTIATION OF IONOTROPIC GLUTAMATE RECEPTOR RESPONSES BY METABOTROPIC RECEPTORS IN THE RAT DORSAL HORN. D. B. Beckmann, R. Smith, F. S. Char, S. R. Glaum & D. M. Miller. Dept. Pharmacol. and Physiol. Sciences, Univ. of Chicago, Illinois 60637. Using both acutely isolated dorsal horn neurons or slices of the adult rat spinal cord (9-16 days) we have examined the effects of the metabotropic glutamate receptor agonist (2S,3R)-ACPD on the 1,3-Dicarbonyl acid (13R)-ACPD (1.5-5M) on responses mediated by the ionotropic glutamate receptor agonist, N-methyl-D-aspartate (NMDA), kainate (KA) and p-amino-3- hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). We measured [Ca2+]i in isolated neurons using flu-2 based microfluorometry and found that in approximately 50% of neurons examined, the (13R)-ACPD (threshold concentration 0.5M) produced increases in the [Ca2+]i, which could be prevented by blockers of voltage sensitive calcium channels. In addition, increases in [Ca2+]i produced by AMPA (10 μM), KA (10 μM), and AMPA (10 μM) were markedly potentiated by (13R)-ACPD (10-30 μM). Potentiation required simultaneous application of both (13R)-ACPD and the ionotropic agonist. However, potentiation was also observed at concentrations of (13R)-ACPD which did not increase responses produced by elevated concentrations of KCl (10-50 mM). (15R)-ACPD potentiated ionotropic responses in all cells examined and reversed rapidly upon washout of (13R)-ACPD.

572.12 INTERACTIONS BETWEEN PHOSPHOLIPASE C-COUPLED AND NMDA RECEPTORS IN CULTURED CEREBELLAR GRANULE CELLS: PROTEIN KINASE C-MEDIATED INHIBITION OF NMDA RESPONSES. M.J. Courtney, K.E.O. Arkoyn, K.M. Popocok and D.J. Nichols. Dept. of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, UK and *Dept. of Biochemistry and Pharmacology, Abo Akademi, SF 20500, Turku, Finland. The NMDA receptor of rat cerebellar granule cells in primary culture is inhibited by phospholipase C-coupled receptor activation. In the absence of NMDA, stimulation of muscarinic, metabotropic glutamate or endothelin receptors induces an elevation of the cytosolic free calcium ([Ca2+]i) monitored with the fluorescent probe fura-2. The response is consistent with the ability of phospholipase C-coupled receptors to release an intracellular pool of Ca2+ and induce subsequent Ca2+ entry into the cell; both responses can be abolished by discharge of intracellular Ca2+ stores with low concentrations of ionomycin or thapsigargin. In cells stimulated with NMDA the [Ca2+]i response to the phospholipase C-coupled agonists is complex and agonist-dependent; however, in the presence of ionomycin each agonist produces a partial inhibition of the NMDA component of the [Ca2+]i rise, activator 45-phospholipid. It is concluded that NMDA receptors in cerebellar granule cells are activated by phospholipase C-coupled receptors via activation of protein kinase C.

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The metabotropic subtype of glutamate receptor in mammalian CNS is thought to be excluded from the synapse, protecting second messenger processes such as an increase of [Ca2+]i and activation of PKC. Here we report that activation of the metabotropic subtype leads to an increase of the ionotropically evoked AMPA and quisquulate responses, whereas NMDA responses are unaffected.

Performance cortex slices (450 µm) were taken from male rats (150-200g) and incubated in Kinger solution for 2h. After transferring the slices into the recording chamber, intracellular recordings were obtained from pyramidal neurons using 60-80 MΩ electrodes filled with 3M KCl (pH 7.2). The metabotropic receptor, 1-10 mM AP-3, and 1 mM NMDA were both applied, whereas AMPA, QUIS, and NMDA were applied iontophoretically (5-20 nA for 10s, 30 s intervals). t-ACPD caused a depolarization accompanied by an increase in input resistance and membrane noise. Although the depolarization was blocked by 10 mM AP-3 and the increase in noise was not. Neither hyperpolarization nor depolarization appeared to alter the noise increase. Surprisingly it is blocked by 1 mM NMDA and this may reflect the involvement of presynaptic Ca2+ channels which trigger other postsynaptic mechanisms. t-ACPD also enhanced the iontophoretically applied AMPA and quisquulate responses, possibly due to increased sensitivity of the AMPA receptor caused by phosphorylation.

Our studies in adult rats and in piriform cortex differ from those reported from neonatal hippocampus. Thus these results may reflect either developmental or regional differences of the metabotropic receptor(s). Supported by NIH NS 28307 (DOC) and the Austrian Science Foundation, “Erwin Schrödinger” fellowship # J-0077-MED (MF).

METABOTROPIC GLUTAMATE RECEPTOR MEDIATED AFTERDEPOLARIZATION IN RAT NEOCORTEXURAL NEURONS. C.C. Green, C.C. Schramm, and W.E. Fishell. Dept. of Physiology & Biophysics, University of Washington, Seattle, WA 98195.

Metabotropic receptors (mGluR) are a family of G-protein linked glutamate receptors. Like muscarinic agonist, metabotropic agonists block the afterhyperpolarization (AHP) in hippocampal pyramidal neurons (Stratton et al. 1989). We used intracellular recording to determine if mGluR mediate a similar effect. The IC50 for the slow AHP (sAHP) of rat neocortical neurons in a submerged brain slice preparation. Slices were bathed in artificial CSF containing kynurenic acid (2-4 mM) or CNQX (50 µM) and APV (50 µM) to block ionotropic receptors, atropine (0.5 µM) to block muscarinic receptors, and s-pentylenetetrazol (15 µM) to block adrenergic receptors. Medium and slow duration AHPs followed 20 action potentials indirectly evoked by current pulses at 100 Hz. When 1-aminocyclopentane-1,3-dicarboxylic acid, 15,30-ACP (14 - 50 µM), a relatively selective metabotropic agonist, was added, a large afterdepolarization (ADP) replaced the sAHP. Glutamate (0.1-1 mM) and quisquulate (0.1 - 5 µM), but not the ionotropic agonist AMPA (0.5 µM), elicited a similar ADP. The ADP represents a new type of mGluR mediated excitatory response which caused persistent self-sustained firing following the cessation of the repetitive firing stimulus in several neurons and contribute to pathophysiological conditions like epilepsy. We have previously shown that muscarinic agonists induced a comparable ADP in cat brain cells. Hence, our findings suggest that the metabotropic receptor activation mimics many of the effects of muscarinic agonists (Miller, R.J., 1991). Supported by PHS grants NS 16792 and GM 07266 and the W. M. Keck Foundation.


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Metabotropic receptors (mGluR) are a family of G-protein linked glutamate receptors. Like muscarinic agonist, metabotropic agonists block the afterhyperpolarization (AHP) in hippocampal pyramidal neurons (Stratton et al. 1989). We used intracellular recording to determine if mGluR mediate a similar effect. The IC50 for the slow AHP (sAHP) of rat neocortical neurons in a submerged brain slice preparation. Slices were bathed in artificial CSF containing kynurenic acid (2-4 mM) or CNQX (50 µM) and APV (50 µM) to block ionotropic receptors, atropine (0.5 µM) to block muscarinic receptors, and s-pentylenetetrazol (15 µM) to block adrenergic receptors. Medium and slow duration AHPs followed 20 action potentials indirectly evoked by current pulses at 100 Hz. When 1-aminocyclopentane-1,3-dicarboxylic acid, 15,30-ACP (14 - 50 µM), a relatively selective metabotropic agonist, was added, a large afterdepolarization (ADP) replaced the sAHP. Glutamate (0.1-1 mM) and quisquulate (0.1 - 5 µM), but not the ionotropic agonist AMPA (0.5 µM), elicited a similar ADP. The ADP represents a new type of mGluR mediated excitatory response which caused persistent self-sustained firing following the cessation of the repetitive firing stimulus in several neurons and contribute to pathophysiological conditions like epilepsy. We have previously shown that muscarinic agonists induced a comparable ADP in cat brain cells. Hence, our findings suggest that the metabotropic receptor activation mimics many of the effects of muscarinic agonists (Miller, R.J., 1991). Supported by PHS grants NS 16792 and GM 07266 and the W. M. Keck Foundation.
PROPERTIES OF A GLUTAMATE AUTOANTEROSEIN IN RAT NEOSTRIATUM D.M. Loogter 

The neostriatum receives a large glutamatergic afferent input from the neocortex. Transmission at striatal glutamatergic synapses is modulated presynaptically by a putative neurotransmitter with metabotropic receptor agonist t-ACPD (Lovinger, Neurosci. Lett., 12, 27-1, 1991). We have examined this receptor using field potential recording from adult rat neostriatal slices and whole-cell recording from rat neurons in slices from 2.4 wk. old rats. In adult slices, a sympathetically-driven population spike (SP) evoked by local electrical stimulation was decreased by bath application of 5-100uM t-ACPD (IC50 of 50uM). This is similar to the potency with which t-ACPD depressed the amplitude of EPSPs in striatal neurons. Depression of the PS was stereospecific with 100uM 135R-ACPD producing an 84-96% inhibition of the response. 135D-ACPD had no effect. The effect of t-ACPD on the PS or EPSP was not altered by the metabotropic receptor antagonist APS (10uM). Phorbol diacetate (PDAc), an activator of protein kinase C, reduced the synaptic depressant effect of 50uM t-ACPD (p<0.005). Alpha-2MA, a phorbol ester which does not activate PKC, did not reduce the effect of t-ACPD (43+5% inhibition of PS in 5uM alpha-2MA). t-ACPD inhibits voltage-activated Ca++ current and activation of PKC inhibits this effect (Szwart & Bean, this meeting). The synaptic depressant effect of t-ACPD may involve an APS-insensitive metabotropic glutamate receptor because these receptors are negatively coupled to voltage-activated calcium channels via PKC-sensitive intracellular processes. Supported in part by grant #A080986 from NIAAA.

PEPTIDES: RECEPTORS V

573.2 IN VIVO INTERACTION BETWEEN NEUROPEPTIDE Y, PEPTIDE YY AND SIGMA RECEPTORS IN THE MOUSE HIPPOCAMPUS. Bouchard, P. 
1,3, Dumas, Y. 1, St-Pierre, S. 2, Fouquet, A. 4 and Quinlin, B. 2,3,4,5,6 (1) Douglas Hospital Research Center, Vancouver, Canada; (2,3)Gatineau, Canada; (4,5)Neurology and Neurosurgery, McGill Univ. and (6) INRS-Sante, Pointe-
Claire, Que., Can.

Recently, it was proposed that neuropeptide Y (NPY) and peptide YY (PYY) could act as endogenous ligands for sigma binding sites since both NPY and PYY compete with high affinity (nM) for [3H]SKF 10407 binding in rat brain membrane homogenates (Roman et al., 1990). However, several laboratories have failed to provide direct evidence for an interaction between NPY and sigma receptors. In order to clarify this apparent discrepancy, we investigated the effects of various peptides on in vivo [3H]SKF 10407 binding parameters in the mouse brain. Mice were injected with NPY (0.1 mg) or PYY (2 mg) subcutaneously or with saline as a specific binding. At 15 min., animal received either a peptide injection (3 ml i.c.v.), or saline. At 30 min., each animal were injected with 5 uCi [3H]SKF 10407 (200 ml i.v). Animal were sacrificed at 60 min. and the binding of [3H]SKF 10407 measured. In the hippocampus, haloperidol competed for up to 90% of [3H]SKF 10407 labelling in a dose of 500 pmol, NPY, NPY-m. (Leu)^3 NPY and PYY competed for 20 to 40% of specific [3H]SKF 10407 binding. Other peptides, like neurotensin and VIP, did not compete with [3H]SKF 10407 binding but surprisingly, CORP competed for up to 30% of the total binding. It thus appears that NPY, PYY and related peptides, as well as CGRP may interact with sigma binding sites in vivo. It now remains to be established if the effects of these different peptides families at sigma sites, are mediated via similar or different mechanisms.


The presence of selective somatostatin (SS) receptor subtypes (SSR1 and SSR2) has been demonstrated in several tissues by means of differential binding potency of octreotide. Both SSR1 and SSR2 bind with high affinity the SS analogs [125I]DTrp[4]SKF[114] (Leu)^3 DTrp[4]SKF[114] in a previous study, we have observed preferential displacement of [125I]DTrp[4]SKF[114] by PYY relative to NPY and other SS receptors. In the current study we characterized these binding sites in the lateral cerebellar nuclei (LCN) by quantitative autoradiography. The binding of [125I]DTrp[4]SKF[114] in the LCN was saturable (Rmax = 219 fmol/mg protein) and of high affinity (KD = 0.5 nM). In contrast, no labeling was detected in LCN using [125I]DTrp[4]SKF[114]. However both S14 and S28 analogs could displace [125I]DTrp[4]SKF[114] and PYY binding, indicating that these sites do not represent SSR preferring receptors. The efficiency of both octreotide and MK 678 in competition tests was very low. The binding of SSR1 and SSR2 with high affinity but did not influence in presence of SSR1 and SSR2. In contrast the competition of SSR2 analogs to compete with the binding of [125I]DTrp[4]SKF[114] in the LCN was: SSR1 (2.0±0.51 mM) and SSR2 (2.08±0.25 mM); [Leu]^3 DTrp[4]SKF[114] (2.08±0.25 mM) and [Leu]^3 DTrp[4]SKF[114] (2.08±0.25 mM); and [Leu]^3 DTrp[4]SKF[114] (2.08±0.25 mM) and [Leu]^3 DTrp[4]SKF[114] (2.08±0.25 mM). A D-Amino acid substitution in position 3, 4, 5, 6, 7, 11, 13, 14, 17, 18, 19, 20, 23, 25, 26, 27 or decreased affinity but had little influence in presence of SSR1 and SSR2. Altogether these results suggest that SSR1 and SSR2 are involved in a structural role and those responsible for receptor recognition.
573.5
UP-REGULATION OF CORTICAL SOMATOSTATIN RECEPTOR BINDING SITES FOLLOWING LONG TERM UNILATERAL LESION OF THE NUCLEUS BASALIS IN THE RAT. D. Closé*, J.C. Martel and P. Doullig. Douglas Hospital Research Center and Dept. of Psychiatry, McGill University, Montreal, Québec, Canada, H3H 1R3.
It has recently been reported that cortical somatostatin-like immunoreactivity (SRIF-IR) is altered following long term uni- or bilateral<br>toxic acid lesions of the nucleus basalis magnocellularis (nBM) in the rat (Arendash et al, Science 238, 1987; ibid, Neurochem. Res., 14, 1989). Earlier<br>data had shown that, contrary to expected results, both SRIF-IR and SRIF receptor binding sites were decreased in Alzheimers brains (Beal et at, Science 229, 1985). Using quantitative in vitro receptor autoradiography, we examined the potential effects of long term changes in cortical NBM levels observed following nBM lesions on its binding sites. Uni- and bilateral<br>toxic acid lesions of the nBM were performed in three-months old rats. Eighteen months later, animals were sacrificed and brain sections prepared for receptor autoradiography. As reported elsewhere (Kramic et al, Neuroscience 39, 1990), [125I-Tyr*]-D-Trp-P-SRPFP ([125I]-SRFPF4) binding sites are concentrated in various cortical laminae, the hippocampal formation and the amygdala body. A long term unilateral nBM lesion induced a significant (compare to sham control) up-regulation of [125I]-SRFPF4 binding in the ipsilateral parietal and temporal cortices while no modification in labelling profile and intensity was detected in the amygdala. Surprisingly, the<br>bilateral lesion failed to induce any significant change in [125I]-SRFPF4 labelling. Thus, as observed for SRIF-IR, cortical alterations of [125I]-SRFPF4 binding can be observed following long term lesions of the nBM but these are<br>dependent on the nature of the lesion performed. Supported by MRC.

573.6
MODULATION OF NEUROPEPTIDE FF RECEPTORS IN RAT BRAIN AND SPINAL CORD MEMBRANES BY GUANINE NUCLEOTIDES AND CATIONS.
K. Payza* and H.Y.T. Yang, LBG, NIMH Neuroscience Center, WAW-113, St. Elizabeths Hospital, Washington, D.C. 20032.
Neuropeptide FF (NPFF) is a mammalian FMRFamide-related peptide of the sequence FLFQFQRamide. NPFF antagonizes morphine analgesia and appears to be involved in morphine tolerance and dependence. In this study, guanine nucleotides, NaCl and MgCl2 were tested for modulatory effects on NPFF receptor binding in membrane preparations of rat brain and spinal cord. Specific binding of [125I]-YLFQFQRamide to NPFF receptors in both brain and spinal cord preparations was stimulated by NaCl and MgCl2 and inhibited by GTP and its nonhydrolyzable analogs. The GTP effect was observed in the presence and absence of Na+ and Mg2+. The specificity of the GTP effect (GTPyS>GPi1>GFPP>GDP, no effect of GMP or ATP) suggests that NPFF receptors are coupled to G-proteins in these tissues. The ionic effects suggest that Na+ and Mg2+ ions are required for maximal receptor binding but not for receptor-G-protein coupling.

573.7
DISTRIBUTION OF NEUROPEPTIDE FF RECEPTORS IN RAT BRAIN AND SPINAL CORD.
Neuropeptide FF (NPFF, FLFQFQR-NH2) is an endogenous neuropeptide with anti-opiate activity. NPFF is unevenly distributed in the rat CNS with the highest concentrations in the spinal cord and hypothalamus. In this study, the distribution of NPFF receptors in rat CNS was examined using radioligand binding assays. The specific [125I-LYLFQFQRamide bindings (displacing proteinᆮpmol total) in various regions of rat CNS were: dorsal spinal cord: 0.123±0.020, medulla oblongata: 0.104±0.008, hypothalamus: 0.099±0.0023, midbrain: 0.0789±0.0086, striatum: 0.0396±0.0035, hippocampus: 0.0163±0.0019, cerebellum: 0.0161±0.0072, cortex: 0.0037±0.0024, ventral spinal cord: 0.023. The radioligand binding in midbrain, medulla oblongata and dorsal spinal cord was inhibited by GTPγS showing that the binding was NPFF receptor specific. The receptor distribution in this study correlated with the NPFF distribution in various regions of brain and spinal cord.

573.8
Rat glioma C6 cells have functional neuromedin B (NMB) receptors. Previously, we reported that NMB bound with high affinity, elevated cytosolic Ca2+ and stimulated the growth of C6 cells in tissue culture. Here NMB receptors were characterized in C6 transplants into the rat forebrain. Cultured C6 glioma cells (104) were transplanted into the rat forebrain. After a postoperative time of 1-3 weeks, the tumors and surrounding host brain were analyzed for NMB receptors and fibroblasts growth factor (FGF) by immunocytochemistry using the PAP method. Antisera to the NMB receptor was elicited against a C-terminal fragment (S-16-L) conjugated to hemocyanin. The NMB receptor antisera strongly reacted with numerous cellular elements but not blood vessels within the C6 glioma. Also, using autoradiographic techniques high grain densities were present for NMB receptors in the graft. In contrast, antisera to FGF strongly reacted with blood vessels within the tumor and adjacent brain. The FGF may stimulate angiogenesis and the NMB receptors facilitate growth within the C6 tumor. Supported in part by NSF grant BNS88-15133 (T.W.M. and NIH grants NS-17468 (J.R) and HL-32348 (T.M.).

573.9
RICIN CYTOTOXIN CELL TARGETING REVEALS UNIQUE ROLES FOR ANP AND CNP IN WATER DRINKING AND PROLACTIN SECRETION. W.K. Sanson* and R.J. Fulton, Anatomy/Neurobiology, U MO Sch Med, Columbia MO 65212 and Inland Labs, Dallas TX 75207.
Centrally administered A-type and C-type natriuretic peptides (ANP and CNP) exert opposite actions on water intake and prolactin (PRL) secretion. While ANP is inhibitory, CNP is stimulatory suggesting actions of the peptides via unique receptors. ANP and CNP were conjugated to the A chain of the plant cytotoxin ricin and conjugates injected icv in adult rats. Two weeks later rats received icv injections of maximally effective doses of either ANP or CNP. In rats pretreated with ANP-ricin A chain, the inhibitory action of ANP on PRL secretion was absent yet the stimulatory effect of CNP was still present. The opposite was true in rats pretreated with CNP-ricin A chain conjugate. Following 18h water deprivation, rats pretreated with ANP-ricin A chain conjugate drank significantly more water than controls, while those pretreated with CNP-ricin A chain consumed significantly less. These results indicate that the opposing actions of these two members of the natriuretic peptide family are expressed via unique receptors, in all likelihood the ANP-R-A and ANP-R-B subtypes.
PEPTIDES: BIOSYNTHESIS AND METABOLISM III

THURSDAY PM

574.1 PRIMARY STRUCTURE OF RF-AMIDE NEUROPEPTIDE PRECURSORS FROM COELENTERATES C. Schmutzler, D. Damer, R. K. Reinscheid, and C. F. Grimmelikhuijzen*. Centre for Molecular Neurobiology, University of Hamburg, Martinistr. 52, D-20000 Hamburg 20, F.R.G.

In the simple and evolutionary old nervous systems of coelenterates neuropeptides are highly abundant and play an important role in neurotransmission and cell-cell signaling. We have isolated a family of neuropeptides with the common C-terminal Arg-Phe-NH$_2$ (RFamide). Thus, these peptides belong to the class of the RFamide peptides which have been found throughout the animal kingdom, the prototype being the molluscan FMRFamide.

We have cloned cDNAs encoding precursor proteins for the RFamide neuropeptide Antho-RFamide (cGluf-Gly-Arg-Phe-NH$_2$) from the anthozoans Anthopleura elegansantana, Calliactis parasitica, and Renilla atlantica which show a highly repetitive organization (containing 13, 19, and >36 copies of immature Antho-RFamide, respectively). We also cloned the precursor proteins for the hydrozoan RFamide heptapeptides Pol-RFamide I and II from Polyorchis penicillus and Hydra-RFamide I, II, and IV from Hydra magnapapillosa.

At the C-terminal side of these peptides precursor cleavage occurs at conventional processing sites (nono-mono dibasic residues). However, we have to postulate "non-classical" mechanisms to explain correct peptide processing at the N-terminal.

574.3 N$^\alpha$-ACETYLATED VasoPressin IN THE RAT PITUITARY GLAND AND HYPOTHALAMUS.

F.M. de Reel, F.W. van Louwe*, and J.H. Burgha.* 1Rudolf Magnus Institute, University of Utrecht, 3521 GD Utrecht, The Netherlands; 2Netherlands Institute for Brain Research, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

Recently N$^\alpha$-acetyl-vasoressin (Ac-VP) and N$^\alpha$-acetyl-oxytocin (Ac-OT) were purified and identified from bovine pituitary gland. Indirect determination of these forms by HPLC combined with a radioimmunoassay using a C-terminal specific VP antisem revealed nanogram amounts of the acetylated forms in the rat neurointermediate lobe (NIL), while the brain itself contained only picogram amounts. The extent of acetylation differed markedly: in the NIL only a few percent of the peptide was acetylated, whereas in the pituitary gland about 70% was acetylated. This indicates a possible regulatory role for this new kind of modification of VP and OT.

In order to analyze and localize Ac-VP directly, anti sera against this peptide were raised against this peptide. Cross-reactivity studies in a radioimmunoassay and dot-blot tests revealed Ac-VP recognizing antibodies with no low cross-reactivity for VP, Ac-OT, OT, N$^\alpha$-acetyl-$\beta$-endorphin and oMSH. One of the antisera (FS5) was used in a radioimmunoassay to determine Ac-VP in NIL and hypothalamus. In the NIL FS5 detected only Ac-VP and no VP indicating a high specificity of FS5. The hypothalamus contained no measurable amounts of Ac-VP. In immunocytochemistry FS5 showed after preadsorption with VP and Ac-OT a fibro-like staining in the posterior lobe. FS5 showed no staining in the hypothalamus after preadsorption. In conclusion VP might be acetylated during axonal transport.

574.4 AN ANTISENSE OLIGODEOXYNUCLEOTIDE INHIBITS PRO-OPINOLIDE RELEASE IN MURINE NEOENDOCRINE CELLS


Gene expression in normal cells can be suppressed by nucleic acid sequences complementary to endogenous transcripts. In actual fact, these "antisense" sequences can hybridize to primary RNA transcripts and prevent translation of the target gene at ribosomal level. We employed this strategy to obtain a significant reduction of the synthesis of peptides derived from the precursor proopiomelanocortin (POMC) in a murine neuroendocrine cell line (AT-20) that highly expresses this gene. Cells grown in suspension in MEM supplemented with 1% fetal calf serum were seeded at 4x10$^5$ cells per dish and exposed to a 30-base pair oligodeoxynucleotide TRACTTOGTCCGATGTCCTCCAGAAAG complementary to mRNA nucleotides which are translated into the first ten amino acids of mouse $\alpha$-endorphin, an opioid peptide derived from POMC. In alternative, AT-20 cells were treated with a sense 30-base pair oligonucleotide. After 24 h cells were harvested and ACTH (another peptide spliced from POMC and positioned upstream to $\beta$-endorphin) was evaluated, by RIA, in acute extracts of the cells. A significant reduction of ACTH content was observed in cells treated with the antisense oligo-nucleotide [26.62 ± 2.9 vs 12.03 ± 0.88 ng/10 cells (n=7) p < 0.01]. These results indicate that antisense oligonucleotides block the synthesis of neuropeptides in cell lines and may be useful in delineating their functions.

574.5 SELECTIVE RELEASE OF THE NEUROPEPTIDE VIP IN SHEEP EXPOSED TO THREE LUNG DAMAGING ORGANOHALIDES: CORRELATION WITH CYCLOOXYGENASE METABOLITES.

R. M. Azzam, 2A. Abraham, 2M. Zetts, 1C. M. Hoedda, 1H. M. van 1M. de Reel, 2U.S. Army Med. Res. Inst. of Chemical Defense, Edgewood, MD 21602; and 2Toxicology Branch, NIEHS, L. N.C. 27790.

Certain neuropeptides, e.g., tachykinins, can promote inflammation while others, e.g., vasoactive intestinal peptide (VIP), may modulate inflammation. We have examined the release of VIP and 3 cyclooxygenase metabolites (CM), after the inhalation of 3 different organohalides that cause lung toxicity. Three groups of sheep (n=10 each) were exposed for 10 min to 323 ppm/min of perfluorobutylcyclohexene (PFB), a pyrrolidine product of Teflon, or 346 ppm/min of bis(tetrafluoromethyl)disulfide (TFD), a pesticide, or 767 ppm/min of phosgene, an industrial chemical. Plasma was collected immediately before and at 15, 30, 60, 120, 180 & 240 min after exposure, and assayed by RIA for VIP and CM in sheep. FG2, TxA2 & F2iso. All three gases produced acute lung injury and elevated CM levels (p<0.05) for up to 60 min. VIP levels with TFD (p<0.05) paralleled those of CM. These data suggest that: 1) the release of VIP was selective, whereas that of CM occurred with three of the gases; 2) release of VIP and CM was both immediate and sustained with TFD and 3) the mechanism of TFD-induced VIP release is unexplained, but it may be an attempt to modulate the injury.

574.6 MICROASSAY OF OPIOID PEPTIDE RELEASE FROM THE NUCLEUS ACCUMBENS AND VENTRAL PALLIDUM OF THE FREELY MOVING RAT M. Braestrup*, C. F. Evans and N.T. Madighan. Department of Psychiatry, NPI and BRI, UCLA, Los Angeles, CA 90024.

We previously described a method for the measurement of opioid peptides in basal ganglia microdialysates of the freely moving rat. However, to carry out such analysis, the animals were required to be naive of the substance under investigation for at least 3 days. This has been possible using [3H]dexamphetamine as the test substance and [3H]dopamine as the labeled neurotransmitter. However, the use of [3H]dexamphetamine is problematic due to the fact that it is a potent stimulant and can increase the activity of the animals. In this study, we have used a modification of the microdialysis technique in which the animals are allowed to move freely and the dialysis probe is implanted in the nucleus accumbens and ventral pallidum. The opioid peptides were measured by microdialysis in the presence of a specific opioid antagonist. The results are shown in Table 1. The opioid peptides measured were mu- and delta-opioid receptors. The mu-opioid receptor was found to be more sensitive to the opioid peptides than the delta-opioid receptor. The results of this study suggest that the opioid peptides are released in a dose-dependent manner in the nucleus accumbens and ventral pallidum. The results of this study also suggest that the opioid peptides are released in a dose-dependent manner in the nucleus accumbens and ventral pallidum. The results of this study also suggest that the opioid peptides are released in a dose-dependent manner in the nucleus accumbens and ventral pallidum.
574.7
SIMULTANEOUS MEASUREMENT OF CCK AND NEUROTENSIN FRAGMENTS IN MICRODIALYSISATES OF THE RAT FOREBRAIN: EFFECTS OF MIDDLE-6-HYDROXYDOPAMINE LESIONS. N. Villarreal, J.D. Barchas* and N.T. Maddox. Department of Psychiatry and Biobehavioral Sciences, N.P.I. and B.R.I., U.C.L.A. School of Medicine, Los Angeles, Calif. 90024.

We previously described a procedure for simultaneous determination of CCK and neurotensin fragments in rat dialysates of the caudate nucleus and posterior nucleus accumbens (Maddox et al., 1991, Neuroscience, 45: 81-93). In view of the well documented localization of these peptides within specific neurons we sought to determine what proportion of the CCK and neurotensin present in these dialysates was secreted by dopaminergic neurones. As a first step in this process we injected 5 rats with 6-hydroxydopamine (10ug in 2ul) unilaterally in the substantia nigra and ventral tegmental area. Four rats received vehicle alone. Four weeks later the animals were used for coanatomical rotational behavior following injection of apomorphine (0.5mg/kg, I.P.). Four weeks following lesioning the animals were reanesthetized and microdialysis was conducted in the posterior nucleus accumbens and medial caudate nucleus ipsilateral to the lesion. Release was evoked by reverse dialysis of veratridine (50nM for 3min) for two 30 min samples separated by 2h. Dialysates were analyzed sequentially for the peptides by solid-phase radioimmunoasssay. Veratridine induced an approximate 10 fold increase in extracellular CCK release in both regions. The neuropeptide response in the nucleus accumbens was smaller (approx 3-5 fold) and less consistent and no stimulation was observed in the caudate. The preliminary data failed to demonstrate a clear effect of the lesion on the release of either peptide in the regions studied. This may reflect a non-dopaminergic origin of the peptides measured extracellularly or a compensatory increase in the releasable pool of the peptide previously reported in the striatum.

Supported by the National Alliance for Research in Schizophrenia and Depression.

574.9
EFFECTS OF LENGTH OF STORAGE AND STORAGE CONDITIONS ON THIOI LEVELS IN RAT SCIAITIC NERVE. K.K. Nickander, N. Lendvai and P.A. Low*. Neurophysiology Laboratory, Department of Neurology, Mayo Clinic, Rochester, MN 55905.

Comparison of the effect of length of storage and storage conditions on rat sciatic nerve levels of reduced and oxidized glutathione (GSH and GSGL, respectively) was reported. With storage in 70% ethanol for 5 days at 0°C, the corresponding ratio was reduced. Two group of rat sciatic nerves were homogenized (in either 0.25 M perchloric acid or 5% 5-sulfosalicylic acid) and centrifuged. A third group was excised and stored unbound immediately in liquid nitrogen. Samples were stored at -70°C for 0, 7, or 26-28 days. The thiols were measured using reverse phase high pressure liquid chromatography and electrochemical detection (Stein et al., 1986). GSH was decreased 56% by 7 days and 72% by 26-28 days while stored in vitro at 0°C. The increase in yield of GSGL was 80% by 7 days and remained high (76%) at 26-28 days. The decrease in the GSH/GSGL ratio was also highly significant at these time points (95% and 94% respectively). The changes were much less significant when stored as unbound thiols.

The levels and ratio remained unaltered when frozen immediately in liquid N2 and stored at -70°C. Therefore, we conclude when storage of rat sciatic nerve tissue is necessary, to freeze immediately in liquid N2 and store at -70°C until analysis. Homogenization should then be done in 5% SSA for best preservation of the thiol levels. (Reference: Stein AF, Dills RL, Klaassen CD. J. Chromatogr. 381:259-270, 1986.)

574.10

To obtain basic information of transglutaminase (TGase) in mammalian brain, a 100,000 x g supernatant fraction was prepared from whole rat brain homogenate. SDS-PAGE analysis followed by immunoblotting of this soluble fraction gave 75 kDa/79 kDa proteins and 48 kDa protein that were detected by polyclonal IgG antibodies against human erythrocyte TGase and guinea pig liver TGase, respectively. However, these immunopositive proteins were catalytically inactive and degenerated from protein with TGase activity by DBAE Sephadex ion exchange chromatography. TGase activity which did bind to the ion exchange column was eluted with 0.5 M NaCl and then applied to a GTP-agarose affinity column. TGase activity was recovered in both the KCl eluate and the GTP-agarose column. The latter fraction was re-applied to the affinity column, the TGase activity was again recovered in the unbound fraction. These results suggest the presence of two different types of TGase in rat brain using the criterion of affinity for guanine nucleotide. It is interesting to note that an immuno- positive 48 kDa protein was also shown to bind GTP.

574.11

Cerebrospinal fluid thyrotropin-releasing hormone (TRH) is elevated in major depression. The metabolism of TRH ultimately yields acid TRH and cyclo-His-Pro (H-Pro) peptides with intrinsic biological activity. Therefore, we have begun to measure cyclo (His-Pro) in affective disorders.

An unselected group of inpatients with major depression (n=5) or bipolar disorder, manic (n=4) was studied during treatment with antidepressants or mood stabilizers, respectively, and while on a low monoamine diet. The mean concentration of plasma cyclo (H-Pro) determined by radioimmunoassay was 2557 +/- 212 pg/ml. This concentration is significantly higher than that reported by Hilton et al (Neuropeptides 13:65, 1989) in ethyndiol control subjects (829 +/- 64 pg/ml, n=14, p<0.0005). No difference between the two patient groups was found.

Limitations and confounds such as varying illness state and treatment make interpretation premature. (Supported in part by the Dept. of Psychiatry, C.U.M.C.)

574.12
COMPUTATIONAL SIMULATION OF NEUROPEPTIDE-LIPID INTERACTIONS: A NEW CONCEPT OF DIRECT EFFECT BY ENDOThELIN-1 AT NEURONAL MEMBRANES. D.F. Weaver, S.J. Farid and P.M. Gros. Department of Chemistry, Medicine, Surgery & Physiology, Queen's University & Kingston General Hospital, Kingston, Canada K7L 3N6

Neuroactive agents may exert their effects on transmembrane ion channels specifically via channel protein receptors or nonspecifically by direct interactions with the neuronal membrane (e.g. local anesthetics). To explore potential membrane interactions for the neuropeptide endothelin-1 (ET), we applied computer-assisted conformational analyses to: 1) ascertain a family of low energy ET conformers, 2) study the effects of different environments on the shape of ET, and 3) simulate ET's interaction with a model neuronal membrane under physiological conditions. Commencing with the NMR conformation of ET, we sought to identify the shape of the molecule having the lowest energy by applying molecular mechanics calculations. MM2, AMBER and DREIDING-2 semi-empirical force-field equations were combined with procedures for both first- and second-derivative energy minimization. Solutions of the lowest energy, thus defined to be a highly flexible molecule, its conformational space was further scanned using molecular dynamics calculations that required several hundred hrs of CPU time on an IBM R6600 S/360 RISC computer. Both aqueous and aqueous-gas environments were simulated at 37°C. Finally, a fully hydrated ET conformer with the lowest energy was positioned adjacent to a phospholipid membrane surface and its molecular motions were studied randomly over a period of 100 sec. These calculations demonstrated that the acyclic hexapeptide tail of ET inserted among the alkyl chains of the lipid membrane in an energetically favorable orientation that affected the shape and dynamic properties of the lipid molecules. The simulation thus revealed a novel mechanism of instantaneous insertion by a neuropeptide into a neuronal membrane. In addition to receptor binding, therefore, ET may invade lipid bilayers directly.

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1369
574.13

**PEPTIDES: BIOSYNTHESIS AND METABOLISM III**

**CCK PARTIAL AGONISTS: DIFFERENTIAL FUNCTIONAL ACTIVITY IN THE RAT.** B. Simon*1, J. Tognone1, B. Julian, F. Kaiser and J. Rossand1. 1Department of Biology and Chemistry, Fisons Pharmaceuticals, Rochester, New York, 14603.

CCK peptide analogs were evaluated for selective CCK-A receptor binding and activity both in vitro using pancreatic amylase release and phosphodiesterase (PDE) inhibition assays, and in vivo, using a 24-hour fasted rat assay. The compounds tested showed a full range of in vitro activity for full agonists to partial agonists as compared to CCK-B. (CCK-Hpa5Ethepe > Boc[Lys4,MePhe7]CCK-4 > Boc[Lys4,Phe7]CCK-4 > CCK-A greater than CCK-B). The feeding-inhibition and antifeedant effects were comparable with the in vitro functional activity which was further correlated with the Ki binding values for the CCK-A, (pancreatic membranes) and CCK-B receptor (cortical membranes). A good correlation exists between CCK-A but not CCK-B binding affinity, in vitro functional potency and in vivo antifeedant potency. However, for partial agonists, in vitro and in vivo efficacy did not correlate. This is probably not related to a difference in receptor subtype interaction (CCK-A vs. CCK-B) since the CCK-A selective agonists (MK-329) but not the CCK-B antagonist (L355,260) completely inhibits CCK induced P.I. turnover and feeding inhibition. Differences in activity could therefore be explained by differences in tissue receptor reserve between the CCK-A receptors in pancreas and those involved in the CCK-B induced feeding inhibition.

574.14

**SUPER-REACTIVITY OF THE SER1 RESIDUE IN GONADOTROPIN-RELEASE INHIBITORY HORMONE AGONISTS IS NOT EVIDENT IN ANTAGONISTS.** B.R. Willers, G. Tsoulfas, T.K. Collins and A. Kurosky. Dept. of Anatomy & Neurosciences and of Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, TX 77555.

The critical roles of gonadotropin-releasing hormone (GnRH) in physiology have led to intensive study of the chemistry, structure and function of this peptide. We have previously demonstrated that the single Ser1 in native GnRH and several GnRH agonist peptides displays unusually high intrinsic reactivity toward activated esters (e.g. TAPS, DSLET, 24) in the rat striatum, 3H-raclopride. However, the functionally analogous Ser1 in native GnRH and several GnRH agonist peptides displays unusually high intrinsic reactivity to activated esters, whereas native GnRH and its agonists were readily O-acylated at Ser1 under identical conditions. These results further emphasize the importance of structure/function considerations of GnRH and its related peptides. (Supported by NIH grant NS 29261 and the Robert A Welch Foundation H-1190)

**OPIN RECEPTORS: INTERACTIONS WITH OTHER SYSTEMS**

575.1

**mu AND K OPIOID AGONISTS ALTER IN VIVO 3H RACLOPRIDE (D2) BINDING: OPPOSING EFFECTS IN THE E1 TAT FETUS AND P10 PUP.** P. Kloth, K. M. Wag1, L. S. Anderson, S. M. Umbersisa, S. R. Robinson2 and W. P. Schmauss.1 1Department of Psychology, Trinity College, Hartford, CT 06106; 2Laboratory of Perinatal Neurochemistry, Center for Developmental Psychobiology, SUNY-Binghamton, Binghamton, NY 13902-6000.

The endogenous opioid system is thought to be in high demand by the dopamine system in the adult; different classes of opioid ligands produce different effects on dopamine. Evidence from in vivo dialysis of adult rat has shown that mu opioid agonists promote dopamine release while K agonists suppress release. Recent developmental studies of neonatal and fetal rats confirm the existence of this opioid-dopamine interaction. The present study employed an in vivo binding technique with a tritiated ligand for the D2 receptor (3H-raclopride) to further examine the development of interactions between the opioidergic and dopaminergic systems in the term fetus (E21) and rat pup (P10). Subjects were injected with mu or k agonists prior to administration of 3H-raclopride. Specific D2 activity was measured in tissue samples from the striatum, septum and hypothalamus. In P10 pups, mu stimulation resulted in decreased raclopride binding while K stimulation increased raclopride binding. In contrast, specific mu binding was increased in specific binding at the D2 receptor in E21 fetuses, while K stimulation produced no change in binding related to controls. These results suggest a developmental discontinuity in the pattern of interaction between opioid and dopamine receptors during the first 1-2 weeks after birth.

This research is supported by Grant RNS 89-13499 (NSF) to PK, Grant HD 16102 to WPS and HD 28231 to WSP and SRR.

575.3

**MU AND DELTA OPIOID-REGULATED ADENYLYL CYCLASE ACTIVITY IN RAT CAUDATE-PUTAMEN AND NUCLEUS ACUMBENCS.** B. Bles, B.Renamecke, and M. Cox.1 Uniformed Services University, Bethesda, MD.

Activation of opioid receptors leads to inhibition of adenyl cyclase activity. Adenyl cyclase activity was examined in crude membrane preparations of nucleus accumbens and the rostral portion of the caudate-putamen using a radioligand binding assay. Basal adenyl cyclase activity (expressed relative to membrane protein) in the caudate-putamen was twice that in the nucleus accumbens. Mu opioid agonists (DAMGO, U69.593) maximally inhibited approximately 30% of basal adenyl cyclase in both brain regions. Both of these mu opioids ligands were less potent for inhibiting adenyl cyclase activity in the nucleus accumbens than in the caudate-putamen. DPDE was equally effective in both brain regions, inhibiting less than 30% of basal adenyly cyclase activity in both regions. In contrast, the non-selective agonists, however, inhibited basal adenyly cyclase activity by 40% of basal cyclase activity in both brain regions. U69.593 had no effect on adenyl cyclase in either brain region. The mu-selective agonist CTOP blocked the inhibition of adenyl cyclase by DAMGO on TAPS, but had no effect on inhibition by DSLET, suggesting that mu opioid receptor activation did not contribute significantly to the inhibition of basal adenyly cyclase activity. When the animals were pretreated with naloxone (30 mg/kg, s.c. 24 hours prior to assay), the effects of DAMGO and TAPS were attenuated but there were no changes in inhibition of adenyly cyclase by DSLET and either DPDE or DPDL. Similarly, 8-Br cAMP (20 µM, 4 hours prior to assay) attenuated the effects of DAMGO and TAPS but had no effect on DSLET. These findings suggest that both DAMGO and TAPS act predominantly through a naloxone-sensitive mu opioid receptor to inhibit adenyly cyclase in these brain regions. Further inhibition of cyclase activity by DAMGO or DPDE, but was not antagonized by any of the mu opioid receptor antagonists. (Supported by a grant from NIDA).

575.4

**REGULATION OF OPIATE RESPONSES IN BRAIN NORADRENERGIC NEURONS BY THE cAMP CASCADE: CHANGES WITH CHRONIC MORPHINE.** R. Shimakawa, K. Asaro-Jones. Department of Mental Health Sciences, Division of Behavioral Neurology, Nahnemann University, Broad and Vine, Philadelphia, PA 19102, U.S.A.

Intracerebro-ventricular (ICV) or intrastriatal injection of morphine (IC stock) or naltrexone (a mu1 antago- nist) into animals pretreated with bilateral injections of ventral mesencephalic dopamine neurons (VIM) and posterior hypothalamic (PH) showed that the ICV administered mu agonists were able to potentiate dopamine release in the basal ganglia. This potentiation was attenuated by the ICV injected mu antagonist naloxone or β-fnaltrexone (a PH injected antagonist). Using immunohistochemical techniques to localize the dopamine release, we have also noted that the ICV injected mu agonist results in a dose-dependent bilateral increase in dopamine release in the caudate-putamen and substantia nigra. These findings have implications for the role of opioid receptors in the regulation of dopamine neurons and their role in the development of tolerance to opiate agonists.

Furthermore, we have investigated the effect of chronic morphine on the ability of mu agonists to potentiate dopamine release in the caudate-putamen and substantia nigra. We have observed a significant increase in dopamine release in the caudate-putamen and substantia nigra of morphine treated animals compared to saline treated controls. This increase was attenuated by the ICV injected mu antagonist naloxone or β-fnaltrexone. These findings suggest that chronic morphine treatment leads to an increase in the sensitivity of dopamine neurons to the action of mu agonists. This increase in sensitivity may be mediated by changes in the expression of opioid receptors or in the coupling of opioid receptors to adenyl cyclase activity.

In conclusion, these findings suggest that chronic morphine treatment leads to an increase in the sensitivity of dopamine neurons to the action of mu agonists. This increase in sensitivity may be mediated by changes in the expression of opioid receptors or in the coupling of opioid receptors to adenyl cyclase activity.
575.5


Effects of opioid agonists and of heterosynaptic preganglionic conditioning stimulation on nicotinic transmission were studied in the superior cervical ganglion (SCG) of anesthetized, paralyzed and artificially ventilated cats. The cervical sympathetic trunk was dissected and split into two bundles, one for conditioning stimulation and the other for testing stimulation. The compound action potential was recorded from the external carotid nerve. A heterosynaptic stimulus train (5 Hz; 40 s) inhibited nicotinic transmission. The inhibition was antagonized by naloxone, suggesting mediation by endogenous opioids. Agonists selective for γ, δ and ε opioid receptors, injected through the lingual artery, also produced naloxone-sensitive inhibition. The synaptic inhibition was occluded by those produced by μ, -ε, but not -selective agonists. The synaptic inhibition was antagonized by the ε-selective antagonist IC 174,864, but not by the δ-selective antagonist Nor-BNI. The ε-selective antagonist CTAP produced a partial antagonism of the inhibition. It is concluded that all three main types of opioid receptors are present in the SCG but only μ and δ receptors are involved in the endogenous opioid-mediated inhibition of ganglion transmission. Supported by the Medical Research Council of Canada.

575.6

INHIBITORY EFFECTS OF OPIOID PEPTIDES ON NEONATE RAT SYMPATHETIC PREGANGLIONIC NEURONS IN VITRO. H. Tan* and N. J. Dun, Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Whole-cell patch recordings were made from sympathetic preganglionic neurons (SPNs) in transverse spinal cord slices and the effects of opioid peptides on these neurons and on synaptic transmission were studied. Two types of responses were detected. First, bath or pressure application of met-enkephalin (Enk 5,10 μM) induced an outward current or hyperpolarization accompanied by increased membrane conductance; the reversal potential was close to E, The δ and µ receptor agonists D-Pen2 and DAMGO and the antagonists IC 174,864 and CTOP mimicked and blocked the response. Second, superfusion of Enk (1-10μM) depressed the excitatory postsynaptic currents (EPSCs) evoked by stimulation of either dorsal roots or lateral funiculus, without affecting the inward current induced by pressure ejection of glutamate. This inhibition of EPSCs could also be mimicked by either the δ or µ agonist. The results indicate that opioids inhibit the activity of SPNs by either a postsynaptic mechanism in opening K channels or by a presynaptic mechanism in reducing the transmitter release. (Supported by NS18710)

575.7

THE SULKING INDUCED PROLACTIN INCREASE IS MEDIATED BY β-ENDORPHIN THROUGH THE KAPPA OPIATE RECEPTOR SUBTYPE. Rebecca Parman, James Janik and Phyllis Callahan*, Miami University, Zoology Dept, Oxford, Ohio 45056.

Administration of β-endorphin to virgin female rats, at doses as low as 25 ng, produced a prolactin secretory response which is the same order of magnitude as the suckling induced prolactin increase. This response was completely abolished by nor-Binaltorphimine (nor-BNN), a specific κ-opioid receptor antagonist. The purpose of this study was to determine whether or not β-endorphin played a physiologically significant role in the suckling induced prolactin increase.

Post-partum lactating female Sprague-Dawley rats were used for all experiments. On day 2 post-partum, animals were implanted with chronic intraventricular (ivt) canulae into the lateral ventricle. Following recovery, each animal was implanted with a chronically catheterized jugular canula. On the day of the experiment, pups were separated from the dams for 6 hours. One group of females received vehicle or Nor-BNN (10 nmol, ivt) 45 minutes prior to pups return. A second group of females received vehicle or antiseraum to β-endorphin (3,7.5 or 15 μg total protein, ivt) immediately prior to pup return. Blood samples were withdrawn immediately prior to and 15, 30, 45, and 60 minutes after suckling was initiated.

Both β-endorphin antiseraum and Nor-BNN pretreatment totally abolished the suckling induced prolactin increase in post-partum female rats. These results indicate that β-endorphin is involved in the suckling induced prolactin secretory response via its activity at the kappa opiate receptor subtype.

575.8

NALOXONAZINE BLOCKS β-ENDORPHIN INDUCED PROLACTIN SECRETION IN FEMALE RATS. James Janik* and Phyllis Callahan, Miami University, Zoology Dept, Oxford, OH 45056.

The prolactin secretory response to β-endorphin administration was determined in post-partum female rats and during the diestrous stage of the estrous cycle. The effects of Naloxonazine (NAZ) pre-treatment were also determined since this μ1 antagonist effectively blocks the morphine induced prolactin increase.

Female Sprague-Dawley rats were used for all experiments. Virgin females in diestrous and post-partum lactating females were implanted with chronic intraventricular (ivt) canulae into the lateral ventricle. Lactating females were implanted on day 2 post-partum. One day prior to the experiment, animals were surgically implanted with a chronic jugular canula. On the day of the experiment, pups were separated from the dams for 2 hours.

The lowest dose of β-endorphin to produce a prolactin secretory response was 25 ng and this dose elicited a prolactin increase which is the same order of magnitude as the suckling induced prolactin increase. This was true in both post-partum female and virgin female rats. Lower doses of β-endorphin, i.e. 2.5, 5 and 10 ng, did not produce a change in circulating levels of prolactin. This prolactin increase was completely abolished by NAZ.

These results indicate that β-endorphin is a potent stimulus for secretion and may act in an "all or none" manner. In addition, it seems that β-endorphin, like morphine, can elicit a prolactin secretory response via its activity at the μ1 opiate receptor subtype.

575.9


The kappa agonists, Cl-977 and PD17302 and the noncompetitive NMDA antagonist MK-801, have been shown to be neuroprotective (reduced LDH release) in primary cortical neuronal cultures treated with exogenous glutamate. These kappa agonists have also been shown to reduce glutamate-stimulated inositol phosphate accumulation while slightly stimulating phosphatidylinositol metabolism when used alone. Glutamate (40 μM) in the presence of phenol red, elevated inositol phosphate accumulation to approximately 400% of basal metabolism, and Cl-977 (50 μM) and PD171702 (30 μM) reduced this response by as much as 47%. It was the purpose of the present study to evaluate whether MK-801 could also reduce glutamate-stimulated inositol phosphate accumulation. MK-801 (50 μM) did not reduce glutamate-stimulated phosphatidylinositol metabolism and did not alter basal inositol phosphate accumulation. However, when given alone at higher concentrations (100-500 μM), MK-801 reduced basal inositol phosphate accumulation by approximately 20-50%. In conclusion, the effects of the kappa opioid agonists and MK-801 are different with respect to basal and glutamate-stimulated phosphatidylinositol metabolism. While Cl-977 may prevent neuronal death, in part, by reducing glutamate-stimulated inositol phosphate accumulation, the only apparent effect of MK-801 was on basal metabolism, implying an interaction between the metabotropic glutamate receptor subtype and the NMDA receptor channel complex.

575.10

MU AND MDELTA INTERACTIONS MEDIATE OPIOID-INDUCED RELEASE OF ADENOSINE VIA N-TYPE Ca2+ CHANNELS FROM DORSAL SPINAL CORD SYNAPTOSOMES. C.M. Callih*, T.D. White and J. Sawynok, Dept. of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

The release of adenine nucleoside (ADN) from the spinal cord dorsal horns is known to be enhanced by spinally administered opioids. This is shown to be a result of spinal microinfusion of morphine (1-20 μM), which has been shown to release ADN from dorsal spinal cord synaptosomes (JPET 243:357, 1989). However, recent studies have shown that the release is mediated by a combination of NMDA receptors and N-type Ca2+ channels.

The present study examined the opioid receptor subtypes involved in the release of ADN from dorsal spinal cord synaptosomes and the Ca2+ channels involved in such release. DAMGO (a δ agonist) evoked an increase in ADN release. DAMGO (a δ agonist) evoked a decrease in ADN release. Both NMDA and N-type Ca2+ channels were involved in opioid-induced ADN release. This study demonstrates that both μ and δ receptors are involved in the release of ADN from spinal cord synaptosomes, and that such release appears to involve Ca2+ entry via activation of N-type Ca2+ channels. (Supported by MRC Canada).
OPIOIDS: ANATOMY AND PHYSIOLOGY II

Thursday PM


we have reported that g opioid receptor activation reduces IPSV amplitudes and increases EPSCs in CA1 pyramidal neurons. However, when the antagomist DPDPE was used EPSPs were increased, spontaneous IPSVs decreased, and evoked IPSVs were unaffected. We concluded that while g opioid receptor activation increased EPSCs in hippocampal neurons, the increase in their amplitude and frequency can be due to a reduction in IPSCs.

Our recent results suggest that this reduction in IPSCs may be mediated by an increase in the number of synaptic contacts of dentate granule cells with CA1 pyramidal neurons. This increase may be due to the decrease in the activity of GABAergic interneurons. We suggest that the increased number of synaptic contacts of dentate granule cells with CA1 pyramidal neurons may be responsible for the decrease in the amplitude of evoked IPSVs.


We have studied in vivo the electrophysiological responses of two selective opioid agonists and systemic naloxone in the dentate gyrus of the hippocampus. D-Ala2, NMe-Bu-NH2 (DAGO), a selective mu receptor agonist, and US50488, a selective kappa receptor agonist, were applied iontophoretically in anesthetized rats. This study represents a continuation of a previous study in which effects of morphine and preliminary data on the effects of locally applied DAGO were reported. The effects of these opioids on evoked field potential activity and on activity evoked by stimulation of the perforant path were recorded. Cells were classified electrophysiologically as either dentate granule cells (DGCs) or dentate interneurons (INTs) using stringent criteria. The responsiveness of the dentate to stimulation of the perforant path was assessed by measuring population spike amplitudes and constructing input/output curves (I/O) and a paired-pulse (PP) paradigm. The effect of naloxone on long term potentiation (LTP) was also studied. There was a tendency for DAGO to produce a decrease in the spontaneous activity of DGCs. This opioid had little consistent effect on the spontaneous activity of INTs; however, DAGO did tend to decrease the number of post-stimulus interneuronal discharges, reduced by perforant path stimulation. As previously reported, DAGO increased the responsiveness of the dentate gyrus to evoked field potential activity. As opposed to DAGO, US50488 was found to have little significant effect on either the spontaneous or evoked activity. Naloxone, administered systemically, readily reversed the effects of DAGO on both single unit firing and on population spikes, and, in preliminary studies, reduced perforant path-induced long term potentiation in the dentate gyrus. (Supported by DA 00145 to JHM).


Our previous pharmacological studies demonstrated that kappa opioid receptor activation inhibited excitatory synaptic transmission in the dentate gyrus. These observations have been extended using whole-cell voltage clamp recordings of dentate granule cells performed in guinea pig hippocampal slices. EPSCs were increased when Co2+ (100 µM) in the recording pipet (to block K+ currents) was replaced with bicuculline (10 µM) in the perfusion buffer to block spontaneous and stimulus evoked IPSPs. A stimulating electrode was placed in the hilar region of the dentate gyrus to evoke EPSCs, which were reduced by the kappa opioid antagonist norbinaltorphimine hydrochloride (100 µM). The effect of this antagonist was reversed by the selective kappa opioid agonist U69,593 (500 nM), a kappa-selective opioid agonist that does not modulate the IPSPs by 45%.

This result suggests that tonic release of endogenous kappa opioids (i.e. dynorphins) also regulate glutamate release from the perforant path. To determine further, a release of additional endogenous kappa opioids, a second stimulating electrode was placed in the hilus to antidromically activate granule cells via their mossy fibers. After establishing a baseline EPSC response, a single high frequency train (10-50 Hz, 1 sec) IPSPs in the hilus resulted in a 15% decrease in the amplitude of the EPSP EPSC. This effect was blocked by naloxone (1 µM) or NBN1 (100 nM). We conclude that the stimulated release of dynorphins from dentate granule cells can act to inhibit EPSCs at the PP synapse. Supported by DOA4123.


We have previously demonstrated a kappa opioid-mediated inhibition of excitatory synaptic transmission in the dentate gyrus of the guinea pig hippocampal slice. In this series of studies we evaluated the effect of kappa opioids on the induction of long-term potentiation (LTP) in this preparation.

Our initial studies defined the stimulus properties of tetanic stimulation sufficient to produce LTP in the dentate granule layer of the dentate gyrus bathed in artificial CSF and 10 µM bicuculline. A tetanus paradigm producing significant but sub-maximal LTP was chosen as the remainder of our studies. In perfused 100Hz trains of 0.3msre 300µA pulses were given at 0 sec intervals and the effect on the population spike amplitude was measured 30 min later. Whereas, this stimulus produced a significant (p<0.01) LTP when compared to uncontrolled tetani, this effect was blocked by pretreatment with 1 µM of the kappa selective opioid agonist U69593. Moreover, pretreatment with the opioid antagonist naloxone (1µM) significantly (p<0.01) enhanced LTP from this stimulus. Thus, kappa opioids appear to inhibit long-term potentiation either when exogenously applied or when released from endogenous stores. Supported by DA04123 and GM70604.


The enkephalins are a class of endogenous opioid peptides thought to be involved in many physiological functions in the nervous system. In particular, many lines of evidence point to their role in the modulation of nociception. In order to assess the effects of enkephalins both in vivo and in culture, and to develop potential therapeutic neuroviruses in modulating nociception, we have developed a series of herpes simplex virus type-1 (HSV) recombinants aimed at expressing the human proenkephalin gene in neural cells following infection. We and others have previously shown that HSV recombinants can be used to confer long term expression of a foreign gene in post-mitotic neurons in culture, or directing the nervous system (Dobson et al, 1991; Andersen et al, submitted).

We constructed the recombinant viruses, the human proenkephalin tDNA, under the control of various promoter elements, was inserted into the coding region of the viral thymidine kinase gene (g4) in a plasmid vector. These transcriptional units were then inserted into the viral genome homologously in a plasmid vector containing viral DNA at the site. Some of the proenkephalin constructs were fitted with a minimal human proenkephalin promoter coupled to one or multiple copies of a CMV/pMC inducible enhancer element. Alternatively, a proenkephalin construct bearing the Moloney murine leukemia virusLTR promoter/enhancer element was also constructed. Different HSV viral genomes have been selected for insertion of the proenkephalin transcript units within their g4 gene: wild-type KOS virus, and also mutant viruses with compromised replication or reduced cytotoxicity. Preliminary results will be presented on the expression characteristics of these viral vectors in cell culture and in sensory neurons in vivo.


This study examines the interactions between estrogen (E), progesterone (P) and a noxious stimulus, 5% formalin (FOM), on preproenkephalin (PPE) mRNA expression in the ventromedial nucleus of the hypothalamus (VMH) and dorsal horn of the lumbar spinal cord (L4-L5) in ovariectomized (OVX) rats divided equally into 3 hormone groups: E+P, E+, and OVX. Rats receiving steroids had 100% E2 artificial implants 2 weeks prior to stimulus injection. P was injected 4 hr prior to sacrifice. Each hormone group, 6 rats received a saline (SAL) injection (s.c.) and 6 received a FORM injection (s.c.) into a hindfoot pad, and rats from all 6 groups were sacrificed 24 hr afterward. Protein sections were hybridized with a single-stranded 32P-DNA probe complimentary to rat PPE mRNA encoding amino acids 6-26. Dissected, dorsal spinal cord(pH8)cDNA was isolated from a minimum of 113 cell/group for the VMH with 2-4 rats/group and from at least 175 cell/group for the L4-L5 segments. At 24 h, no difference in PPE mRNA levels were seen between sides or between SAL and FORM injections in either the DII or VMH. 6 OVX rats that received no injection have been added as a control stimulus. So far, analysis of mean pixels (labeled in lumen and cell of the DH from 2-3 cell group shows no apparent effect of steroid treatment or injection alone but suggests a trend for an interaction between these factors (p=0.07). Similar analysis of the VMH data from 2-4 rats/group shows an effect of hormone treatment (F=7.87, p=0.002) but no apparent effect of injection solution or interaction between hormone and injection. In the VMH, post-hoc Tukeys show that the mean pixels/cell are greater in both E and E+ than in OVX rats (p=0.05). Mean pixels/cell are greater in OVX- FORM than OVX-SAL rats, suggesting that when PPE mRNA levels are low in the VMH, as in OVX rats, FORM can increase PPE transcription.
KAPPA OPIOID RECEPTION OF THE SECRETION OF PROLACTIN AND 2-
MELANOCYTE-STIMULATING HORMONE IN MALE AND FEMALE RATS. J.
Manzanares and J. Rodriguez-Manzanares. Department of Pharmacology &
Toxicology, Michigan State University, MI 48824.
Previously studies from our laboratory have demonstrated a sexual difference in
the responsiveness of melanocyte-stimulating hormone receptor kappa opioid
receptor agonists and antagonists (Neuroendocrinol. 55: 301, 1992), but it is
not known if this difference is reflected by similar differences in the secretion of
prolactin. The aim of the present study was to examine the effects of the
kappa opioid agonist U-50,488 and antagonist nor-binaltorphimine (NOR-
BNI) on the secretion of prolactin in male and oestrus female rats. For
comparison, the effects of U-50,488 and NORTHR by the intravenous adminis-
tration of a-MSH were also examined. On the day of the experiment, rats were
implanted with right atrial cannula under disheveler anesthesia and, and serial blood samples were taken -30, 0, 30, 60,
90, 120, 240, 480 min relative to drug activation. Administration of kappa opioid
receptors with U-50,488 (5 mg/kg ip) caused a marked and time-dependent (90-120 min) increase in plasma prolactin concentrations in female rats, but produced only a transient increase in prolactin secretion in males. Blockade of kappa opioid receptors with NOR-BNI (25 mg/kg ip) had no effect on plasma prolactin concentrations in female rats, but produced a pronounced and time-dependent (90-480 min) decrease in prolactin secretion in males. These results reveal a sexual difference in kappa opioid receptor-mediated regulation of prolactin secretion. In contrast, there was no sexual difference in kappa opioid regulation of a-MSH secretion since administration of U-50,488 increased a-MSH decreased prolactin concentrations in both male and female rats. (Supported by ADAMHA Grant MH 42802).

ESTROGEN AND PROGESTERONE MODULATION OF AN
INTRINSIC OPIOID ANALGESIC SYSTEM(S). M.E. Dawson-Baoa* and A.R. Gintzler. Dept. of Biochemistry, SUNY Health Science Center at
Brooklyn, N.Y. 11203, USA.
It has been demonstrated in rats as well as in humans, that pregnancy
and parturition are associated with an opioid-mediated elevation in
mammary gland pain threshold. This analgesia has been shown to involve a spinal opioid system(s). Simulation of the pregnancy blood profile of 17-
β-Estradiol (E2) and Progesterone (P) in non-pregnant, ovariectomized rats resulted in statistically significant elevations in pain threshold. The
increase occurred at effects of E2 and P that occurred during late pregnancy (1-3 days before birth) and parturition, the time of actual pregnancy during which analgesia is also observed. Administration of pregnancy levels of P alone or E2 with the delayed addition of P (starting with dose 3, the dose at which initial increases in pain threshold occur) is not sufficient to produce the increase in pain threshold. Therefore, the entire pregnancy profile of steroid hormones is responsible for the manifestation of analgesia. Administration of the narcotic antagonist naloxone blocked the increase in pain threshold achieved during hormone-simulated pregnancy, indicating that it is mediated via an endogenous opioid system(s), as is the analgesia of actual pregnancy. The striking similarity between the analgesia of hormone-simulated pregnancy and actual gestation strongly suggest that the profile of change in plasma E2 and P are parameters of the pregnant condition essential to the manifestation of elevated pain thresholds.

ESTROGEN RAPIDLY ATTENUATES THE RESPONSE OF GUINEA
PIG HYPOTHALAMIC NEURONS TO μ-OPIOIDS. A.H. Lagrange,
O.K. Ronneklev and M.J. Kelly*. Department of Physiology, Oregon Health
Science University, Portland, OR 97201-3098.
Neurons of the hypothalamic arcuate nucleus are hyperpolarized by μ-
opioids via an inwardly-rectifying potassium conductance. Ovariectomized
guinea pigs (GPs) treated with estrogen 24 hours prior to sacrifice show a
rightward shift in dose-response curves with μ-opioid agonists, DAMGO, versus opioid antagonists. There is no change in the maximal
response to DAMGO or the K, for antagonism by naloxone. Presently, we have made intracellular recordings in hypothalamic slices prepared from ovariectomized GPs to show a more rapid effect of 17-
b-estradiol (E2). Dose-response curves to DAMGO, followed by washout of the opioid and perfusion with 200mM E2 for 20 minutes showed the same three-fold rightward shift when a second dose response curve to DAMGO was performed (p<0.001, n=8). The E2Cm shifted from 80 ± 16mV to 236 ± 67mV. This effect did not appear to be homologous desensitization by DAMGO as multiple dose-response curves, both before and after estrogen, did not show this shift. Moreover, the change in sensitivity to DAMGO was seen in slices treated with E2, without prior exposure to exogenous μ-opioids. The effect of E2 could be seen for up to 3 hours after washout of this steroid. Histochemical double labelling identified a subpopulation of these cells as α-endorphin neurons. These actions of E2 imply a direct effect on the receptor-G protein-K+ channel effector system. (Supported by PHS grants DA05158 & HD00718).

INHIBITION OF THE TIME-DEPENDENT INWARD RECTIFICATION IN
VASOPRESSIN CELLS OF THE GUINEA PIG SUPRAOPTIC
NUCLEUS BY THE MU OPIOID AGONIST DAMGO. K.B. Erickson*,
Dept. of Physiology, Oregon Health Sci. U., Portland, OR 97201-3098.
Guinea pig magnocellular neurosecretory cells (MNCs) exhibit a time-
dependent inward rectification in the cell from hyperpolarized membrane potentials (below -65 mV). This increase the excitability and, in vasopressinergic (AVP) neurons, leads to phasic firing (Erickson, et al., Soc Neurosci. 1991). In the present study, the μ-opioid agonist DAMGO (300 nM) was bath-applied to AVP-supraopti-
uc nucleus (SON) MNCs. Intracellular recordings were made in an in
vitro slice preparation using bicuculline-filled electrodes, thus permitting
immunocytochemical identification. Six cells were identified as AVP-
containing. Two others were not AVP positive. Five of the six AVP cells responded to DAMGO (0.5 - 1 μM), whereas neither of the two AVP-
negative cells responded (p<0.05). All five responding cells exhibited a TDR in current clamp or an I, in voltage-clamp. DAMGO hyperpolar-
ized and inhibited the TDR in current clamp (N=2); and in voltage clamp, DAMGO produced an outward current and inhibited the I, on steady-state ramp IV measurements (N=2). Naloxone (1 μM) depolarized the membrane by 2 mV and increased the TDR in an AVP neuron from a morphine-treated guinea pig, indicative of an antagonism of tonic opioid tone. The results suggest that the TDR (I, in guinea pig AVP MNCs is modulated by μ-opioid receptor activation. (Supported by PHS grant DA05158).
576.15

POSSIBLE INTERACTION OF \(\mu\)-OPIOD AND \(\alpha_2\)-ADRENOCEPTORS IN DERMORPHIN-INDUCED BRADYCARDY IN CONSCIOUS RATS.

O. M. Adeyemo and A. L. Sirin*, Dept. of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Picolome doses of the selective \(\mu\)-opioid agonist dermophin (DM) increased mean arterial pressure (MAP) and heart rate (HR) after intracerebroventricular (i.c.v.) administration by a \(\mu\)-opioid receptor mechanism while naloxonazine (DM i.v. induced bradycardia which was related to activation of \(\mu\)-opioid receptors (Paikari P. K. et al., Neuropharmacol. 1992). Bradycardia is also associated with stimulation of central \(\alpha_2\)-adrenoceptors. In the present study the influence of \(\alpha_2\)-adrenoceptor on the cardiovascular effect of DM i.c.v. was examined in conscious male Sprague-Dawley rats (250-300 g, n=30) using the \(\alpha_2\)-agonist clonidine (CLDN) and SKF 84646 (6-chloro-N-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine), a selective non-opioid \(\alpha_2\)-adrenoceptor antagonist (Hsieh J.P. et al., J. Pharm.Exp. Ther. 236: 90-96, 1986). In order to block any \(\mu\)-opioid receptor mediated effects, naloxonazine (NLZ), a \(\mu\)-antagonist, (30 \(\mu\)g/rat, i.c.v.) was administered 20 minutes before the interactions between DM and the drug were tested. DM (3-10 nmol/rat, i.c.v) induced a dose-dependent bradycardia in NLZ treated rats. SKF 84666 (1 \(\mu\)mol/rat, i.c.V. 30 min before DM) had no effect on MAP and HR but blocked the bradycardic effect of DM (3-10 nmol/rat, i.c.v). CLDN (10 \(\mu\)g/rat, i.c.v. 30 min before DM) had no effect on heart rate and did not modify the DM-induced bradycardia. These data suggest that \(\alpha_2\)-adrenoceptors interact with the \(\mu\)-opioid receptor associated bradycardia.
CATECHOLAMINES:

The activation potentiation.

To selectively activate the lateral PP input to dentate gyrus stimulating electrodes were placed in the lateral olfactory tract (LOT). Single LOT pulses were used to initiate field EPSPs in the dentate gyrus and were preceded by PGi pulses at intervals which would result in coincident release of NE and lateral PP glutamate. PGi activation consistently reduced EPSPs (see graph) and also reduced EPSPs evoked by stimulating lateral PP fibers directly. PGi stimulation in the same experiments potentiated the medial PP population spike. The data suggest LC-NE is a highly selective promoter of input-evoked responses in the dentate gyrus.


5-HT neurons in the dorsal raphe nucleus (DRN) are shown to inhibit noradrenergic neurons terminating in the hypothalamus (Eton et al., 1992). In the present study, we evaluated the role of 5-HT neurons in the regulation of NE and DA neurons in the MZI and DMN in rats. Catecholaminergic neural activity in these brain regions was estimated by measuring the accumulation of 3,4-dihydroxyphenylalanine (DOPA) after administration of a dopamine agonist and the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), indices of synthesis and metabolism, respectively. Injection of 5-HT neurons with 5-HT, against 8-hydroxy-2-(D-ala-5)-propionylaminovaleric acid (8-OH-DPAT) increased the accumulation of DOPA in the DMN and the concentration of DOPAC in the MZI and DMN, indicating an activation of either NE or DA neurons in these regions. The results of the present study reveal that 5-HT neurons tonically inhibit NE neurons terminating in the MZI and DMN, but not the catecholaminergic DA neurons located in these regions. (Supported by NIH grant NS15191).

5-HT 4.07:5.7-1991).

The medial zona incerta (MIZ) and dorsomedial nucleus of the hypothalamus (DMN), which contain cell bodies of the laterodorsal tegmental nucleus (DLi), are innervated by noradrenergic (NE) neurons and 5-hydroxytryptaminergic (5-HT) neurons. The purpose of the present study was to examine the role of 5-HT neurons in the regulation of NE and DA neurons in the MZI and DMN in rats. Catecholaminergic neural activity in these brain regions was estimated by measuring the accumulation of 3,4-dihydroxyphenylalanine (DOPA) after administration of a dopamine agonist and the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), indices of synthesis and metabolism, respectively. Injection of 5-HT neurons with 5-HT, against 8-hydroxy-2-(D-ala-5)-propionylaminovaleric acid (8-OH-DPAT) increased the accumulation of DOPA in the DMN and the concentration of DOPAC in the MZI and DMN, indicating an activation of either NE or DA neurons in these regions. The results of the present study reveal that 5-HT neurons tonically inhibit NE neurons terminating in the MZI and DMN, but not the catecholaminergic DA neurons located in these regions. (Supported by NIH grant NS15191).

5-HT 5.7-6.07:5.7-1991).

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CATECHOLAMINES: NOREPINEPHRINE

THURSDAY PM

Society for Neuroscience Abstracts, Volume 18, 1992

577.9

COMPARISON OF AIMA MAX AND DIETHYLDITHIOCARBAMATE (DDC) ON RAT HYPOTHALAMIC MONOAMINE LEVELS. N. J. Chang1, C. A. Barb1, L. S. Lesher1, J. B. Hampack1, B. Johnson1, E. B. Krawlig1 and L. J. Wright2
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DDC reduces brain norepinephrine (NE) content and subsequent LH secretion in the rat. AIMA MAX, also a carbamate compound, suppresses LH secretion. However, the mechanism of AIMA MAX action is unknown. Concentrations of monoamines in the hypothalamus were compared after DDC or AIMA MAX in ovariectomized rats primed with an injection of 50 μg estradiol benzoate followed 48 h later by 2.5 μg progesterone (d 0) sc. Treatments were: 1) injection sc of 650 mg DDC/kg bw (n=6) on d 0; 2) daily injection of 20 mg AIMA MAX/kg bw (n=6) from d 0; 3) saline (C; n=6). Injections were given between 1000 and 1030 h. Rats were decapitated on d 0 between 1530 and 1630 h. Monoamine content and reticular areas (RCA) were determined. AIMA MAX reduced (P<0.05) NE and increased (P<0.05) dopamine (DA) content in MSH and RCA compared to C rats. Similar patterns in NE and DA levels were observed in DDC rats. However, DDC elevated epinephrine (EPI) content in MSH and RCA compared to AIMA rats. Therefore, like DDC, AIMA MAX is an apparent NE synthesis inhibitor.

577.11

EFFECTS OF S-2-(3-METHYLAMINOPROPYLAMINO) ETHYLPHOSPHOROTHIOIC ACID (WR-3869) ON THE CONTENT OF CATECHOLAMINES IN MOUSE ADRENALES. D.I. Palazolo1, W.A. McLean and K. Sree Kumar1, Radiation Biochemistry Department, AFRI, Bethesda, MD 20889.

Simultaneous administration of caffeine and WR-3869 mitigates impairment of locomotor activity in mice treated with WR-3869 alone. This combination of drugs also decreases the adrenals. Mice were treated with saline (control) or WR-3869 (100 or 200 mg/kg), adrenals removed at 0, 1, 2, 4, and 8 hr after injections and dopamine (DA), norepinephrine (NE) and epinephrine (EPI) determined using HPLC. Treatment with 100 mg/kg WR-3869 had no effect on DA; 200 mg/kg WR-3869 increased (p<0.05) DA content from a control value of 29±6 to 37±5 mg/g wet weight 1 hr after treatment. With both 100 and 200 mg/kg WR-3869, NE content decreased (p<0.02) from 66±4 to 39±6 and 43±8 mg/g, respectively 4 hr after treatment. EPI content decreased (p=0.04) from 117±8 to 85±12 mg/g 4 hr after treatment with 100 mg/kg WR-3869, but increased (p<0.03) from 131±2 to 174±11 mg/g 1 hr after treatment with 200 mg/kg WR-3869. These results indicate that WR-3869 alters catecholamine metabolism.

577.13

THE EFFECTS OF CHRONIC OPiate TREATMENT ON [3H]norepinephrine UPTAKE PROPERTIES OF NORDADERGIC LOCUS COERULEUS NEURONS IN VITRO. H.A. Raymond* and F.M. Leslie. Dept. of Pharmacology, University of California, Irvine, CA 92717.

Endogenous opioids have been implicated in the control of developmental events, such as cell proliferation and differentiation. The cell types that are under opioid control during development have not been clearly identified. The purpose of this study was to determine whether the maturation of norendnergic locus coeruleus (LC) neurons is influenced by chronic opioid treatment. LC cells were obtained from the rostral rhombencephalon of rats at embryonic day 14. Cells were dissociated and plated in either a serum-containing or serum-free, fully defined (NE) uptake was used as a marker for the growth of norendnergic cells in culture. LC cells treated chronically with fentanyl citrate for 4 days in serum-containing medium showed a significant decrease in [3H]NE accumulation. The dose-response curve was U-shaped, with a maximal effect at 10 nM and a reduced effect at the highest dose (1 μM). Fentanyl inhibition of [3H]NE uptake was reversed by the opiate antagonist, naloxone. Kinetic constants derived from uptake velocity curves for control and drug-treated cultures were: Km = 206 ± 38 nM and Vmax = 53 ± 8 fmol/min/mg and Km = 120 ± 10 nM and Vmax = 89 ± 13 fmol/min/mg, respectively. This indicates a change in the affinity of the NE transporter with chronic opiate treatment. No significant differences in uptake were found in fentanyl-treatned cultures grown in defined medium. The mechanism of the effect in defined medium may be due to the development of tolerance. Experiments utilizing opioid inhibition of [3H]NE release as an index for functional opioid receptor activity in serum-containing and defined medium to determine whether tolerance develops after chronic fentanyl treatment. Overall these data suggest that chronic opiate treatment influences monoamine transporter functions in developing LC neurons. Supported by NIH grants NS19319 and MOH9737.

577.10


To study the rapid influence which a static magnetic field has upon brain metabolism by means of in vivo microdialysis, dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillie acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine (NE) in a rat caudate putamen were monitored during the application of a 250 gauss static magnetic field, applied to a whole body by a 2 tesla (T) NE superconductive magnet. A 28 week old male Winter rat (n=7) was placed in a block cloth-covered free moving unit while monitoring. After 2 hours of monitoring, in 15 minute intervals, during the application of the magnetic field, the changes in monoamine levels were not significant when compared to normal changes in the absence of the field. By using a static magnetic field of 250 gauss, rapid influence was not determined by changes in monoamine levels in the rat caudate putamen. In response to the report that the B.B. barrier function is altered by a magnetic field, the change may not have been rapid or it may not have had a direct influence upon the monoamine levels in caudate putamen. If a magnetic field dose have an influence on the brain metabolism, not only for monoamine levels in the caudate putamen but also other metabolites, it may appear after long time monitoring or in the presence of a stronger magnetic field.

577.12

INTRACEREBROVENTRICULAR INJECTION OF ANGIOGENIN II INCREASES PLASMA NOREPINEPHRINE LEVELS. B. Koga1, M. I. Phillips1 and C. Sumners, Dept. of Physiology, University of Florida, Gainesville, FL 32609.

Centrally mediated effects of Angiotensin II (All) include increases in blood pressure, drinking and release of vasopressin (AVP). The increase in blood pressure has been postulated to occur both through the increase in AVP and an increase in peripheral sympathetic activation. We investigated the effect of intracerebroventricular (icv) injections of All on the release of peripheral norepinephrine (NE) and epinephrine (E) in Sprague-Dawley male rats. NE and E were measured by radioenzymatic assay. To determine whether release of NE from the adrenal played a role in the response NE in plasma was measured after adrenalectomy. The result shows that icv. injections of All (50-500 in 100 μg) caused a significant increase in plasma NE levels. The highest dose of All (500 μg) also caused an increase in E levels. Adrenalectomy lowered E concentrations to below the detection limit of the assay. The All-stimulated increase in NE levels persisted in the adrenalectomized rats, and at a dose of 250 μg All i.e. the effect was not significantly different from intact animals. However, at 500 μg All i.e. this response was significantly attenuated. The results confirm that central All releases NE and E peripherally. The source of E appears to be by stimulation of the adrenal gland, but high doses of All are required. The source of NE is the peripheral sympathetic nervous system at low doses and the adrenal medulla at the high dose.
STUDIES ON THE COORDINATION REGULATION OF GENES ENCODING TYROSINE HYDROXYLASE AND TETRAHYDROBIOPTERIN (BH4) BIOSYNTHETIC ENZYMES IN CULTURED PC12 CELLS. P. Z. Anastasio and R. A. Levine. Lab of Molecular Neurobiology, Lafayette Clinic, and Cellular and Clinical Neurobiology Program, Wayne State University, Detroit, MI, 48007.

Tetrahydrobiopterin (BH4) is the naturally occurring cofactor for tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), which are the initial and rate-limiting enzymes in the catecholamine (CA) and serotonin (5HT) synthetic pathways, respectively. Genetic deficiencies in BH4 biosynthesis occur in neurologic disorders, including Parkinson disease (PD) and 5-HT deficiencies. The BH4 synthetic pathway is regulated by protein kinase B (PKB) in the nervous system, which is known to affect the CAergic neurotransmitter system.

The purpose of this study was to identify the regulatory mechanisms affecting BH4 biosynthesis in the nervous system. This study examines the regulation of BH4 biosynthesis in the nervous system using a cell culture model. The effects of PKB on BH4 biosynthesis were assessed in PC12 cells, which are a clonal rat pheochromocytoma cell line that overexpresses TH and TPH. The effects of PKB on BH4 biosynthesis were assessed using a luciferase reporter assay and measuring BH4 levels in the media. This study found that PKB phosphorylation of the BH4 synthetic enzymes increased BH4 biosynthesis, indicating that PKB plays a key role in regulating BH4 biosynthesis in the nervous system.

The results of this study suggest that PKB regulates BH4 biosynthesis in the nervous system. These findings provide insights into the regulation of BH4 biosynthesis in the nervous system and may have implications for the treatment of neurodegenerative disorders and psychiatric diseases.
CATECHOLAMINES: TYROSINE HYDROXYLASE

578.7 ELECTROCONVULSIVE SHOCK INCREASES TYROSINE HYDROXYLASE AND NEUROPEPTIDE Y MESSENGER RNA IN THE LOCUS COERULEUS. S.-Y. Fang, T.S. Haycock, J.D. Silverman, A. Galli, R.H. Reith, Deps. of Pathology, Beth Israel Hospital and Harvard Med. Sch., Boston, MA 02215 and Deps. of Pharmacology and Microbiology, Yale U. Sch. of Med., New Haven, CT 06511.

Tyrosine hydroxylase (TH) is encoded by a single gene in humans, but four distinct mRNA transcripts are generated by alternative splicing from a single transcript. In humans, there are two types of TH mRNA transcripts; the protein isoform of TH is synthesized from the longer transcript. TH messenger RNA (mRNA) has been reported to exist in the brain. As part of our effort to study the development of TH messenger RNA (mRNA) in the brain, we have examined the expression of TH mRNA transcripts in brains of embryonic, fetal and adult African green monkeys. Total RNA extracts from adult monkey tissues and fetal brain were analyzed by northern blotting and reverse transcripted and the cDNAs were further amplified by PCR techniques using a pair of primers based on human TH sequences. In adult monkeys, four distinct PCR fragments were obtained. The four types of TH transcripts were detected in adrenal medulla and in midbrain regions such as the substantia nigra and the ventral tegmental area. The observed sizes of the PCR products were identical to those predicted for different human TH mRNA subtypes. DNA sequencing of these PCR fragments is in progress. In developing fetal monkey brains, TH transcripts were barely detectable at 42 and 59 days, but at 81, 91, and 150 days after gestation, four types of TH mRNA species were detected in the midbrain regions. In contrast to previous findings, these results indicate a lack of divergence in TH mRNA splicing pattern among primates, and that in the monkey, four types of TH mRNA transcripts are present in brain tissue as well as in adrenal medulla. Further studies are in progress to study the expression of some of the neurotrophic factors and their receptors whose actions may influence the development of the mesencephalic DA neurons. Supported by AI-22674, AI-23990, MH-14092 and the Axton Research Foundation.

578.8 LOCUS COERULEUS MESSENGER RNA ABUNDANCE IN VIVO IN THE RAT SUBSTANTIA NIGRA AND MAMMALIAN CORPUS CALLOSUM. B.S. McEwen and E.A. Stone Lab. of Neuroendocrinology, The Rockefeller University and Department of Psychiatry, NY University Medical Center, NY, NY 10021.

Recent work has suggested that the central nervous system is involved in long term adaptive responses of the central nervous system to stress. It has also been found that the dopaminergic (DA) system may play an important role in the stress response. A number of studies have shown that lesions to the midbrain, the substantia nigra, are produced by the experimental procedure of lateral brain lesions. Since the midbrain is involved in the regulation of the response to stress, the present study was designed to further elucidate the NA-DA interactions by examining the effects of unilateral lesions of the LC on containing neurons in the SN. Unilateral LC lesions were produced stereotaxically by injection of 2 µl (2mg/ml) 6-OHDA into the right LC. Seven days after the lesion, the animals were injected with yohimbine (5mg/kg) to release NA from non-lesioned terminals, so as to accentuate the difference in NA function between the two sides. Animals were sacrificed after 2 hours. Immunohistochemical assay using a monoclonal antibody against tyrosine hydroxylase (TH) revealed marked reduction in immunoreactivity in the midbrain in the lesioned side. In situ hybridization using oligonucleotide probes for TH mRNA demonstrated a significant reduction of TH mRNA in the ipsilateral side. These results were especially dramatic in the anterior portion of the SN pars compacta. Thus, the NA system may exert a supportive and facilitative effect on DA substantia nigra neurons. Supported in part by: AG389 82-028R, MH45265, MH41256.

578.9 EFFECTS OF PREFRONTAL CORtical LESIONS ON TYROSINE HYDROXYLASE GENE EXPRESSION IN MIDBRAIN DOPAMINE NEURONS. H. E. Nye and A. Y. Deutch, Departments of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510, and VA Medical Center, West Haven, CT 06516.

Retrograde tracer studies have demonstrated innervation of the ventral tegmental area (VTA) from a variety of sites, including the medial prefrontal cortex (PFC). In particular, the infralimbic (IL) and ventral prelimbic (PL) parts of the PFC project to the VTA. Both of these PFC areas increase Fos expression in response to stress, while other afferents to the VTA do not. In particular, stress increases dopamine (DA) metabolism and release in the PFC and VTA, and since ibotenic acid lesions of the PFC prevent the stress-induced increase in DA release in subcortical sites, similar lesioning of the PFC may reduce functional activity of certain midbrain DA neurons and may alter tyrosine hydroxylase (TH) gene expression. We therefore examined the effects of PFC lesions on TH mRNA in the VTA. The IL and PL were lesioned by microinjection of 8-OHDA. TH mRNA levels in the lesions of the PFC were persistence in selected animals served as two types of controls. Animals survived for either 5-6 or 21-22 days before being sacrificed. TH mRNA was determined by Northern blotting.

Lesions of the PFC involved the IL and ventral PL in all cases; in some cases lesions extended dorsally. Lesions of the PFC did not appear to alter TH mRNA in the total VTA (21 days survival). In situ hybridization studies are in progress to determine if PFC lesions alter TH mRNA in a restricted portion of the VTA, i.e., in those cells projecting to the PFC. Supported by MH-43124, TG GM-07324, and the VA National Centers for Schizophrenia and PTSD at the West Haven VA Medical Center.
Catecholamines: Tyrosine Hydroxylase

578.13

Previously we have reported that antibodies to a synthetic peptide, TH-16, corresponding to a serine (Ser)-40-containing fragment of TH recognize catecholaminergic neurons and processes in the CNS (Lee, K. Y., et al., Neurochem. Sci. 1990, 998, 451-24). We have now generated polyclonal antibodies to TH-16 phosphorylated at Ser-40 by protein kinase A and designated them as anti-TH-16-P. Anti-TH-16-P recognizes the phosphorylated form of nonphosphorylated state of TH. Thus, a positive ELISA response against phosphorylated TH purified from PC12 cells and a negative response against the nonphosphorylated enzyme were obtained. Immunohistochemical results in a decrease of TH activity of the phosphorylated form, but not of the nonphosphorylated. Immunohistochemical studies reveal that anti-TH-16-P recognizes noradrenergic and adrenergic neurons of the mesolimbic and medullary. It also recognizes a small subset of midbrain dopamine (DA) neurons, staining some neurons in the ventrolateral tegmental area and in the posterior aspects of the A10 cell group. However, other DA neurons including the A9 (SN) and A8 (retrobulbar) cell groups were not, or very weakly, immunoreactive. In the forebrain there was weak or absent staining in the neocortex and the thalamic nuclei. These results suggest that the phosphorylated form of TH recognized by anti-TH-16-P is present in relatively high concentrations in noradrenergic, but not in abundance in dopaminergic cell groups. These studies were supported in part by NIH 5R03 and NIH 5R01.

578.15

Although b.c. cerebellar (CB) and the neurointermediate lobe (PIT) are densely innervated by catecholaminergic fibers, catecholamines or their biosynthetic enzymes have not been previously detected in the perikarya of adult CB or PIT tissue. However, we have recently detected mRNA for the biosynthetic enzyme, tyrosine hydroxylase (TH), in CB and PIT of adult male Sprague Dawley rats. Total RNA was extracted from these regions using a guanidinium isothiocyanate (GITC). TH mRNA was converted into single stranded cDNA using a specific downstream primer and then subjected to polymerase chain amplification (PCR) in the presence of oligo primers 5' and 3' to the 12th codon of the TH cDNA. PCR products were analyzed by gel electrophoresis, transferred to nylon membranes and probed with a 32P-labeled oligo complementary to nucleotides 1233-1267 of the TH cDNA. Specific amplification products ranging in size from 127-429 bases were produced when various combinations of 4 different primers were used. Because the primers recognized sequences lying on different exons, products amplified from genomic DNA vs cDNA could be easily distinguished. The origin of TH mRNA in these regions is presently unknown. Based on preliminary non-radioactive in situ hybridization experiments it is possible that this message is contained within axons innervating these structures. Studies are in progress to evaluate the validity of this hypothesis and to examine whether levels of TH mRNA in the CB or PIT can be regulated by pharmacological stimuli.

578.16

Catecholaminergic neurons and processes in the CNS (Lee, K. Y., et al., Neurochem. Sci. 1990, 998, 451-24). We have now generated polyclonal antibodies to TH-16 phosphorylated at Ser-40 by protein kinase A and designated them as anti-TH-16-P. Anti-TH-16-P recognizes the phosphorylated form of nonphosphorylated state of TH. Thus, a positive ELISA response against phosphorylated TH purified from PC12 cells and a negative response against the nonphosphorylated enzyme were obtained. Immunohistochemical results in a decrease of TH activity of the phosphorylated form, but not of the nonphosphorylated. Immunohistochemical studies reveal that anti-TH-16-P recognizes noradrenergic and adrenergic neurons of the mesolimbic and medullary. It also recognizes a small subset of midbrain dopamine (DA) neurons, staining some neurons in the ventrolateral tegmental area and in the posterior aspects of the A10 cell group. However, other DA neurons including the A9 (SN) and A8 (retrobulbar) cell groups were not, or very weakly, immunoreactive. In the forebrain there was weak or absent staining in the neocortex and the thalamic nuclei. These results suggest that the phosphorylated form of TH recognized by anti-TH-16-P is present in relatively high concentrations in noradrenergic, but not in abundance in dopaminergic cell groups. These studies were supported in part by NIH 5R03 and NIH 5R01.

578.14
EVIDENCE OF PRE OR POST-TRAINING ACUTE ADMINISTRATION OF 8-OH-DPAT IN ASSOCIATIVE LEARNING. A. Nunez* and E. Humes. Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología, CIENVEST-IPN, México, D.F.

Diverse evidences suggest a role for 5-HT1A receptor agonists in learning and memory. In the present work, it was determined the pre or post-training injection (ip) of 8-OH-DPAT (DAPAT) on autoshaping lever press response, a model for associative learning. Animals were individually trained to find 15 US in the food magazine. Once that the animal ate the USs, one session began. Each session consisted of 20 trials and each trial consisted of 15 retractable levers for 8 sec (conditioned stimulus, CS) followed by the delivery of a food pellet (unconditioned stimulus, US) each 60 sec. If the animal pressed the lever (conditioned response, CR) the trial was shortened and the lever was retracted, the light was turned off and US was delivery. The results showed the consolidation of CR when this was injected post-training, but impaired it when administered before training. Both effects were time-dependent. When DPAT was injected pre or post-training to free-feeding or pre-feeding rats, they did not learn the CR. When DPAT was administered to overtrained animals (90-100% of CR) the compound did not elicit any effect. These results strongly suggest an effect of DPAT on the consolidation of learning. The pre-training administration impaired the learning but also the food intake and exploration.

SEROTONIN RECEPTORS: BEHAVIORAL ACTIONS

579.1
FURTHER EVIDENCE FOR 5-HT-1C-MEDIATED POTENTIATION OF AMPHETAMINE-PHINUCATED LOCUMOTOR ACTIVITY. P.B. Hicks*, R.J. Zavodny and K.A. Young. Department of Psychiatry, Scott and White Clinic; Department of Medical Pharmacology and Toxicology and Department of Medical Anatomy & Neurobiology, Texas A&M College of Medicine, Temple, Texas 76508.

As reported previously (S. Neurosci. Abstr. 17, 407), the 5-HT1C antagonist mesulergine potentiated apomorphine-induced locomotor activity (AILA) at a low dose that had no effect on spontaneous locomotor activity. DOI, which has affinity for 5-HT1C receptors as an agonist, reversed this potentiation. The current study further characterizes this putative 5-HT1C effect. Doses of mesulergine significantly lower than the AILA potentiating dose of 0.1 mg/kg SC had no effect on AILA, while mesulergine doses including and higher than 0.5 mg/kg suppressed AILA. This information suggests that mesulergine maximally potentiates AILA between 0.05 and 0.3 mg/kg. The TH1 antagonist ketanserin and MLDL 100,907, tested over a wide range of doses, suppressed AILA at higher doses but did not potentiate AILA at low doses. Furthermore, ketanserin did not reverse AILA by the relatively specific 5-HT2 receptor antagonist MLDL 100,907. These findings provide further evidence that mesulergine's potentiation of AILA is a 5-HT1C mediated effect and suggest an important modulatory role for 5-HT1C receptors in motor behaviors associated with DA neurotransmission.
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**579.3**


A recent investigation indicated that there is an interaction between 5-HT1A and 5-HT7 receptors at the neuronal level in the medial prefrontal cortex (mPFC). In this study, we examined the head-twisting (HTW) response induced by injection of 8-OH-DPAT in rats pretreated with the 5-HT1A receptor agonist WAY-100635. While the HTW response was significantly reduced by WAY-100635, it was not eliminated. These results suggest that there is an interaction between 5-HT1A and 5-HT7 receptors in the mPFC.

**579.4**


Recent data obtained from behavioral and radioligand binding studies suggest that there is an interaction between 5-HT1A and 5-HT7 receptors in the rat brain. In this study, we examined the interaction between 5-HT1A and 5-HT7 receptors in the mPFC of adult male Sprague-Dawley rats. This was accomplished using the techniques of single cell recording and iontophoresis. The iontophoresis of the 5-HT1A receptor agonist 8-hydroxy-d-(-)-propylaminotetralin (8-OH-DPAT) produced a current-dependent suppression of mPFC cell firing. This effect was selectively antagonized by the 5-HT7 receptor antagonist NON-190 but not by the antagonists eticlopride (D2), atenolol (β), prazosin (α1), lidoxan (α2), and prazosin (5-HT1A) or SR 91131 (GABA(B)). The suppressant action of DPAT was prolonged and potentiated by the iontophoresis of the 5-HT1A/5-HT7 receptor antagonist ritanserin and the selective 5-HT1A antagonist (−)-MDL 11,939. This systemic administration of ritanserin (0.1-0.3 mg/kg, i.v.) also potentiated and prolonged DPAT's suppressant action. In contrast, neither the ionophoretic nor systemic (0-1.0 mg/kg, i.v.) administration of gabapentin potentiated or prolonged DPAT's suppressant action. The iontophoresis of a low current of the 5-HT2A/5-HT7 agonist (−)-DOI potentiated l-glutamate (GLU)-induced excitation of mPFC cells. This effect was significantly attenuated by the ionophoresis of DPAT at currents that had little or no effect on L-GLU-induced excitation alone. Overall, these results indicate that there is an interaction between 5-HT1A and 5-HT7 receptors at a neuronal level.

**579.5**

Characterization of phosphoinositide phosphatase accumulation induced by the 5-HT7 receptor agonist 2-methyl-5-HT. W.Y. Wang, C.R. Ashby, Jr, and R.W. Wang. Department of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY 11794.

The aim of the present study was to examine the effect of manipulations of calcium (Ca2+) concentrations and activation of protein kinase C (PKC) on 2-methyl-5-HT-induced phosphoinositide (PI) hydrolysis in the rat medial prefrontal cortex. The omission of Ca2+ from the Krebs incubation medium significantly reduced the 5-HT1A-induced inositol phospholipid accumulation from pre-labeled phospholipids (from 41 ± 3% to 23 ± 1%), which was abolished by co-incubation with 5-80 mM phorbol 12-myristate 13-acetate (PMA), but not by the inactive isomer 4a-phorbol, as an activator of PKC. These results suggest that 2-methyl-5-HT is a Ca2+-dependent process. Furthermore, the process is subject to a negative feedback inhibition via PKC. (Supported by USPHS grants MH-41440 and DA-07193).

**579.6**

Food intake, locomotor and temperature effects of 5-HT7 antagonists and agonists. Pascale Mazzola-Pumettto, Charron, R., Aukhil, Mary L., Misra, and Daniel H. Wein. Department of Neurological, Clinical Science, National Institute of Health, Bethesda, MD 20892.

We studied the effects of various doses (0.1, 1.0 and 10.0 mg/kg) of MDL-72222 (a 5-HT7 antagonist) and (1S,2R-2-phenyl)-4-benzylidene (a 5-HT7 agonist) on food intake using food-deprived paradigm, locomotor activity and rectal temperature in male Wistar rats. Administration of MDL-72222 produced dose-dependent suppression of food intake. However, statistically significant decreases were observed with 1.0 and 10.0 mg/kg doses but not with 0.1 mg/kg dose. On the other hand, only the highest dose (10.0 mg/kg) of MDL-72222 produced significant decreases in locomotor activity. Administration of various doses of (1S,2R-2-phenyl)-4-benzylidene did not have any significant effect on food intake except for a small (24%) nonsignificant decrease at the highest dose (10 mg/kg) during the first hour. However, there was a significant increase (56%) in food intake during 14 hours with the highest dose only. These findings suggest that the locomotor effects of the 5-HT7 antagonists and agonists may partially contribute to their effects on food intake. We will also present data on changes in rectal temperature following administration of various doses of 5-HT7 antagonists and agonists.

**579.7**


There is a lack of reliable animal models to screen in vivo 5-HT1D ligands. Recently, the hindlimb scratching (HS) induced by serotoninergic compounds in rats has been suggested as a model for peripheral 5-HT1D-like receptors. In this study we have compared the HS inducing properties of the non-selective 5-HT1D agonist 5-hydroxytryptophan (5-HTP; 5-HT-5.1), 5-methoxytryptamine (5-MeO-5-HT; 5-HT-5.2) and serotonin (5-HT; 5-HT-5.3) as well as the selective 5-HT1D agonist LY335979 (5-HT-5.4) and the 5-HT1D antagonist ketanserin (5-HT-5.5). The results indicated that all 5-HT1D agonists induced a significant increase in scratching frequency, with LY335979 having a more pronounced effect (51%) and 5-HTP a 10% effect. Moreover, ketanserin reduced the 5-HT (5%) and the 5-MeO-5-HT (10%) induced HS. These results suggest that HS induced by ketanserin may be a 5-HT receptor-mediated response.
EVIDENCE FOR INVOLVEMENT OF 5-HT2C AND 5-HT7 RECEPTORS IN THE FOOD INTAKE SUPPRESSIVE EFFECTS OF 1,2-DIMETHOXY-4-IODOPHENOL-2-AMINOFROPANE (DOI); JAMES L. HILL, CHARKAIT, S. AULAKH, AUDREY RIEG*, AND DENIS L. MURPHY, (Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892)

We studied the effects of various 5-HT receptor subtype selective antagonists on DOI-induced decreases in food intake using the food-deprived paradigm in male Wistar rats. The effects of the antagonists when administered alone were also examined. Administration of DOI to rats produced dose-related decreases in one-hour food intake. Pretreatment with m-chlorophenylpiperazine (m-CPP), a 5-HT2 antagonist, completely blocked whereas mesulergine, mianserin and ritalinserin (5-HTc/5-HT2 antagonists) partially blocked DOI's effect on food intake. In contrast, pretreatment with m-chlorophenylpiperazine (m-CPP) significantly potentiated DOI-induced suppression of food intake. Furthermore, the food intake suppressive effects of various doses of DOI were found to be similar in the Fawn-Hooded (FH) rat strain as compared to the Wistar rat strain. These findings suggest that DOI-induced suppression of food intake is mediated by stimulation of both 5-HT2C and 5-HT7 receptors.

SEROTONIN RECEPTOR MODULATION

5-HT1 receptors alter the repetitive firing activity in vagal motoneurons through modulation of T-type calcium currents. L.J. molenaar, B.J. koppen, and M.B. denke, School of Medicine, University of Kentucky, Lexington, KY 40506-0253.

In a previous study, we demonstrated that serotonin (5-HT) reduces spike frequency adaptation (SFA) and increases the post-burst hyperpolarization (PBH) observed in motoneurons from the dorsal motor nucleus (DMX) of the vagus nerve (Moelnas and denke, Neurosci. Abstr., 17: 545-9, 1991). In this report, we have used a heparin saline preparation (400 um thick) from adult guinea pigs to study the specific serotonergic receptor subtype responsible for this effect. Bath applied guanidium dinitrate (GDN, 50 uM), an agonist for 5-HT, and 5-HT receptors, did not affect either SFA or the PBH. In addition, serotonergic modulation of SFA and the PBH were not affected by the 5-HT1 and 5-HT7 antagonist cyproheptadine HCl (40 uM), but 2-methylserotonin isoleucine (2-M-5-HT) (50 uM), a selective agonist for 5-HT2 receptors, reduced SFA and increased the PBH with a similar magnitude and time course of action as 5-HT. Both the 5-HT1 and 2-M-5-HT reduction of SFA and augmentation of the PBH were effectively blocked by the selective 5-HT1 antagonist MDL 72222 (30 uM). We have also attempted to further characterize the ionic mechanism underlying the action of 5-HT. Addition of 300 mM NaCl to the bath had no effect on SFA or the PBH in control solutions but did block the effects of 5-HT and 2-M-5-HT. In contrast, 300 mM CaCl2 in the bath completely eliminated SFA and the PBH. In the presence of both 5-HT and CaCl2, SFA and the PBH returned. These data suggest that 5-HT modulates the repetitive firing activity of vagal motoneurons through a 5-HT2 receptor subtype acting on a NMDA sensitive conductance such as T-type calcium channels. (Supported by NIH grant HL40386, HL02314, and RR07114).

GABAERGIC HETERORECEPTORS AND 5-HT1B AUTORECEPTORS MEDIATE THE OXMETAZOLINE-INHIBITED SUPPRESSION OF RAT VENTRAL HIPPOCAMPAL 5-HT RELEASE IN VIVO; S. Hjorth* AND R. Toghi, Department of Pharmacology, University of Goteborg, Medicinare, 7-541 90 Goteborg, Sweden

There is evidence that both 5-HT1B autoreceptors and GABA receptors participate in the control of transmitter release from 5-HT nerve terminals in vivo (Hjorth & Toghi, J. Pharm. Pharmacol., 34: 209: 249, 1991; Toghi & Hjorth, NSAP 345: 137, 1992). However, the subtype of GABA autoreceptor involved in this action has not been defined. We have used the 5-HT1B autoreceptor selective agonist oximetazoline (OXY) to further characterize the mechanism(s) regulating 5-HT release in vivo. Studies were carried out by means of in vivo microdialysis (probes placed in the ventral hippocampus) in chloral hydrate-anaesthetized male Sprague-Dawley rats (250-350 g). Drugs were introduced via the perfusion medium after a control period of 2.5 hr (to establish baseline 5-HT output). OXY (0.3-10 uM) concentration-dependently suppressed the 5-HT output (max. drop = 40-50%). The effect of OXY (10 uM) was abolished by co-perfusion with the 5-HT1B receptor blocker methiothepin (1 uM) plus the non-selective GABA autoreceptor blocker mazdaxan (10 uM), but at best partially antagonized by either drug given alone. The GABA autoreceptor antagonist mazdaxan (10 uM) did not affect the OXY response. The OXY-induced reduction of the 5-HT release in rat ventral hippocampus in vivo appears to be due to the combined 5-HT1B and GABA autoreceptor agonistic properties of the compound, thus supporting the idea that there is a functional synergism between 5-HT- and NA-mediated control of 5-HT release. In this regard, the results of the present study suggest that the 5-HT1B autoreceptors involved are GABA autoreceptors situated on the 5-HT neuronal terminals.
**SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 10, 1992**

**S.0.5**

**CHRONIC ADMINISTRATION OF THE 5-HT1A RECEPTOR AGONIST 8-OH-DPAT PRODUCES DESENSITIZATION OF 5-HT1A AUTORECEPTORS.** L. Lucke and D. S. Kreiss, Dept. of Psychiatry and Pharmacology, Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

The functional mRNA for 5-HT1A autoreceptors was determined by measuring the ability of the 5-HT1A receptor agonist 8-OH-DPAT to reduce the release of serotonin (5-HT) in the striatum and the hippocampus using in vivo microdialysis. Locomotor activity was measured in the ventral caudate nucleus and the ventral hippocampus of rats maintained under chloral hydrate anesthesia. Acute systemic administration of 8-OH-DPAT (1.0 mg/kg, s.c.) reduced the release of 5-HT in both the striatum and the hippocampus. Chronic treatment with 8-OH-DPAT (10.0 mg/kg, s.c.) for 7 days attenuated the effect of an acute challenge with 8-OH-DPAT in the striatum. Treatment with 8-OH-DPAT for 1 day did not significantly affect the levels of the agonist challenge on striatal 5-HT release. In contrast, the inhibition of 8-OH-DPAT release in the hippocampus by 8-OH-DPAT was not altered by chronic treatment with the 5-HT1A receptor agonist for 7 days. These results indicate that in vivo microdialysis can be used to study the effects of activation of 5-HT1A autoreceptors on 5-HT release in vivo. The ability of 8-OH-DPAT to inhibit release by chronic administration of psychoactive drugs. Moreover, this study indicates that the regulation of 5-HT release in different brain regions by 5-HT1A autoreceptors can be determined by analysis and characterization of the 5-HT1A receptor agonist 8-OH-DPAT. Supported by USPHS grants MH 36262 and MH 48125.

**S.0.6**


The potential anxiolytic effects of BMY 14802 were determined in a modified rat social-interaction paradigm. Unfamiliar pairs of male rats were placed into a novel high-light area. Locomotor activity was measured concurrently with observation of socially interactive behaviors (e.g., mutual grooming and sniffing, following). BMY 14802 (0.25-1.0 mg/kg, p.o.) produced dose-dependent increases in locomotor activity with minimal effect on locomotor activity. Increases were comparable to those produced by buspirone (0.025-1.0 mg/kg, p.o.). In rats undergoing withdrawal following cessation of subchronic diazepam administration for 7 days, buspirone (0.5 mg/kg, i.p.) failed to increase time spent in social interaction, whereas BMY 14802 (0.1 mg/kg, i.p.) and ondasetron (0.1 mg/kg, i.p.) retained their anxiolytic-like effects. The data indicate that BMY 14802 produces anxiolytic-like effects in the rat that are maintained under conditions of diazepam withdrawal; these effects may arise from weak partial agonist actions at 5-HT1A receptors.

**S.0.7**

**IMMUNOEXOGENOMIC CHARACTERIZATION OF RECOMBINANT MOUSE TRYPHTHAN HYDROXYLASE EXPRESSED IN E. COLI.** D.R. Park, D.K. Bemotas, M.H. McDonald, J.I. Color, T.H. Nielsen, and D. Goldman, Lab. of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892.

Tryphtphan hydroxylase (TPH) is the first and rate-limiting enzyme in serotonin biosynthesis. In order to produce the large amount of purified TPH for the purpose of biochemical characterization and antibody production, recombinant DNA technology was used. In the present study a mouse cDNA containing the full coding region for TPH was cloned by the polymerase chain reaction, using poly A+ RNA prepared from mouse dorsal raphe nuclei and oligonucleotide primers. The primer construct was expressed in an expression plasmid under control of the tac promoter. Cultures of E.coli host strain M15 were grown overnight in a liquid medium containing ampicillin, with 2 mM IPTG and pelleted by centrifugation. Large quantities of enzymatically active recombinant TPH were expressed in a highly insoluble form. Moreover, more than 75% of total enzyme activity could be solubilized by lysis and repeated sonication. A band corresponding to TPH protein was eluted from SDS-polyacrylamide gels and extensively dialyzed to remove from buffer through a 50000 dalton dialysis membrane. Immunoblotting of TPH was performed using anti-TPH antibodies. The results indicate that TPH is expressed in a human brain. These methods can be used to produce large quantities of enzymatically active TPH. Supported by MH4403.

**S.0.8**

**A 5-HT2A AGONIST FOR THE TREATMENT OF MIGRAINE: PHARMACOLOGICAL STUDIES WITH MDL 100,687.** J. Spreuse, C. Schenilli, C. Tsigou, R. Hammerschmidt, I. Reske, M. D. Dudley, I. McDonald, J. S. Davis, S. P. S. F. Pak, and M. Reeh, Merrell Dow Research Institute, Cincinnati, OH and Strasbourg, France and Montreal Neurological Institute, Montreal, Canada.

The classification of 5-HT2A receptors into at least four subtypes tantalizingly suggests specific targets for drug therapy. Yet, with the exception of 5-HT2A receptors in the treatment of anxiety, the designation of a "disease for every subtype" remains unrealized. Enantiomeric (SUM), which is effective in abolishing migraine attacks, is often described as acting at "5-HT-sites". In the present study, MDL 100,687 (MDL, 4-[2-(5-hydroxy-1H-indol-3-yl)ethylaminol]-4-[3-(4-trifluoromethylpheny1)hexamidine, monydrochloride), a novel compound targeted for migraine, was characterized in various assays of 5-HT2A specificity and function.

MDL-bound to 5-HT2A sites in bovine caudate membranes with an IC50 of 34 mM compared to 81 mM for SUM. In guinea pig cortical slices, MDL inhibited K+-induced release of [3H]-5-HT by activating terminal 5-HT2A receptors; IC50 for MDL was 123 nM and for SUM, 1002 nM. MDL suppressed forskolin-stimulated adenylate cyclase in guinea pig substantia nigra with an IC50 of 388 nM vs. 1910 nM for SUM. In functional tests relevant to the treatment of migraine, both MDL and SUM reduced cat carotid arterial blood passing through arteriovenous anastomoses (MDL, 66% decrease at 1 mg/kg i.v.; SUM, 48%) and MDL dose-dependently constituted human pial arteriole (pD2 = 7.14 for MDL and 5-HT). The results indicate that MDL is clearly a 5-HT2A agonist; its relative efficacies in migraine awaits clinical trials.

**S.0.9**

**DIRECT SEQUENCE AND SSCP ANALYSIS OF THE HUMAN TRYPHTHAN HYDROXYLASE GENE.** J. E. Ellision, D. A. Nielsen, C. J. McHugo, and D. Goldman, Lab. of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20892.

Tryphtphan hydroxylase (TPH) is rate-limiting for the synthesis of serotonin, a neurotransmitter whose function may be perturbed in several neuropsychiatric diseases with a genetic component. The human cDNA sequence and mouse genomic DNA sequence, including intron/exon boundaries, were utilized to prepare primers for the PCR amplification and automated sequencing of the TPH gene from human genomic DNA. The exon portions of ten overlapping PCR fragments were sequenced and also compared by the single strand conformational polymorphism (SSCP) method. Intron 7 was sequenced and the mutation nature of a polymorphism detected by the SSCP method and located within this intron was determined. For SSCP analysis for mutations, DNA segments ranging from 121 to 3310 bp in length were amplified with fluorescent dye-labelled primers and the larger fragments were enzymatically digested prior to nondenaturing electrophoresis. Fragment mobility variants were argon laser detected using an ABI 373A Sequencer. Primers were designed for PCR amplification and sequencing were delivered from individuals low in C5F-5 hydroxindolacetic acid, an index of serotonin turnover, from individuals with behaviors correlated with altered serotonin function (including alcoholics, murderers, firestarters, and suicide attempters) and from normal individuals.

**S.0.10**

**REGULATION OF 5-HT2 RECEPTOR mRNA LEVELS BY 5-HT2 RECEPTOR AGONISTS AND ANTAGONISTS IN CULTURED CEREBELLAR NEURONS.** J. Akoshki, C. Hough, and D. M. Chang, Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

We have studied effects of 5-HT2 receptor agonists (5-HT, DOI) and antagonists (mianserin, ketanserin) on 5-HT2 receptor mRNA expression in cerebellar granule cells cultured in vitro for 8 days. Pretreatment with 5-HT or DOI induced a rapid desensitization of 5-HT2 receptor-mediated 5-HT response and a subsequent increase of 14C-ketanserin binding to 5-HT2 receptors in crude membranes and intact cells. The increase in 5-HT2 receptor binding sites was dependent on the concentration of the predesensitizing agonist and associated with an increase in both 5-HT and K+-evoked 5-HT release. Moreover, the up-regulation was temporally correlated with a decrease of 5-HT receptor mRNA level (from 24 hr after treatment). Total RNA and mRNA for m1-muscarnic receptors and a-actin were not differentially affected by 5-HT or DOI pretreatment. Conversely, exposure to the 5-HT2 antagonists mianserin and ketanserin induced a time-dependent decrease of 5-HT receptor mRNA levels. Thus, in cerebellar granule cells, the number of 5-HT2 receptors and 5-HT2 receptor mRNA level are regulated by 5-HT2 receptor agonists and antagonists in an unusual manner.
The regulation of serotonin (5-HT) release by somatodendritic 5-HT₁A autoreceptors was examined using in vivo microdialysis. 5-HT release was measured in the ventral caudate nucleus and the ventral hippocampus of rats maintained under sodium chloride hydrate anesthesia. Systemic administration of 8-OH-DPAT (1.0 mg/kg, i.p.) completely reduced the release of 5-HT in both the striatum and the hippocampus. Infusion of 8-OH-DPAT (1.2 µg in 25 µl over 5 min) into the doral raphe nucleus completely inhibited 5-HT release in the striatum, but did not alter 5-HT release in the hippocampus. Conversely, infusion of 8-OH-DPAT (1.2 µg in 25 µl over 5 min) into the dorsal hippocampus completely inhibited 5-HT release in the striatum, but not in the striatum. Systematic microinjection of the antagonist (-)-propranolol (5.6 µg in 25 µl over 5 min) into the dorsal raphe nucleus with systemic administration of 8-OH-DPAT (1.0 mg/kg, i.p.) completely blocked the inhibition of 5-HT release in the striatum without affecting the inhibition of release in the hippocampus. Conversely, simultaneous microinjection of propranolol into the dorsal raphe nucleus with systemic 8-OH-DPAT blocked 5-HT release from being inhibited in the hippocampus, but not in the striatum. These results indicate that 5-HT release in certain areas of the striatum and the hippocampus are differentially regulated by 5-HT₁A autoreceptors of the hippocampus and the dorsal raphe nucleus.

Supported by USPHS grants MH 36262 and MH 48125.

Differential regulation of serotonin (5-HT₁A) subtypes and the involvement of therapeutic agents with a serotonergic mechanism of action (serotonin reuptake inhibitors as antidepressants; 5-HT₁A agonists as anxiolytics and antidepressants; a 5-HT₁A agonist as an antimigraine agent) have kindled interest in novel 5-HT₁ agonists. We describe a new N,N-diarylpropylamine (DAP) derivative, 5-(5-aminomethyl-2-thiazolyl)-DMT (CP-110,330), that exhibits potent binding affinity (IC₅₀ in parenthesis) for 5-HT₁A (3.0 nM), 5-HT₁B (6.6 nM), and 5-HT₁D (3.0 nM) receptors with weak binding to 5-HT₁C (1.0 µM) and 5-HT₁F (14 µM) with an IC₅₀ of 0.55 µM (125I)DIO receptors. Agonist (5-HT₁A) character of CP-110,330 is suggested by a Gpp(NH)p shift to lower affinity in 125Iodocyanopindolol binding. In addition, CP-110,330 shows marked inhibition of DA uptake (IC₅₀ 0.11 µM; cf. mazindol). IC₅₀ 0.14 µM. The high binding affinity for 5-HT₁A sites is readily rationalized, because CP-110,330 is a C₅-substituted DMT. The DA uptake blocking activity, however, is more difficult to explain. The conformation of CP-110,330 may allow the terminal phenyl ring and the anisomorphyl sidechain to approximate a phenethylamine-like or a phenylbutylamine-like conformation with binding to the DA uptake site (IC₅₀ 0.6 nM in 1H[3]HTPC). The SAR of these analogs appears to be consistent with this possibility.

Two kyurenine aminotransferases (KAT I and KAT II) are capable of producing the neuroactive tryptophan metabolite kynurenic acid from L-kyurenine in human brain tissue. We have now purified KAT I to homogeneity and characterized its catalytic properties. The enzyme was purified approximately 2,000-fold with a yield of 2%. Assayed by polyacrylamide gel electrophoresis, KAT I migrated to a single band as a single protein with a mobility of 0.5. The pure enzyme is a dimer consisting of two identical 57 kDa subunits. Among oxo acid tested, KAT I showed no activity with 2-oxoacrycates. Using this co-substrate, kinetic analyses revealed an apparent $K_m$ of 1.8 $\mu$M for L-kyurenine. KAT I activity was potently inhibited by glutamate, phenylalanine and tryptophan. Anti-KAT I antibodies were produced and partially purified. Subsequent Ouchterlony double diffusion, immunoblotting and immuno blotting analyses confirmed that KAT I is distinct from other known kyurenine aminotransferases. Taken together, pure human KAT I and its antibody can be expected to serve as valuable tools in future studies of kyurenine acid production in the human brain under physiological and pathological conditions. (cf. Jauch et al. this meeting).

This work was supported by USPHS grant NS 28236.

**581.3** PURIFICATION AND CHARACTERIZATION OF MEMBRANE ASSOCIATED GLUTAMATE DECARBOXYLASE FROM PORCINE BRAIN. N. Nathani, K. Bao, and J.-Y. Wu. Dept. of Physiology & Cell Biology, Univ. of Kansas, Lawrence, KS 66045-2106

Several lines of evidence point to the conclusion that multiple forms of L-glutamate decarboxylase (GAD), the synthetic enzyme for GABA, are present in mammalian brain. Here, we describe purification and characterization of a membrane associated GAD, referred to as mGAD, from porcine brain. The purification involved solubilization of mGAD with 0.5% Triton X-100, followed by column chromatography on anion exchanger, DEAE-53. Three mGAD activity peaks were obtained, one of which was further purified to homogeneity by additional column chromatography on hydroxyapatite, Blue Sepharose Q-200 and preparative SDS-PAGE. The molecular weight of native mGAD was calculated to be 125 ± 10 kDa from gel filtration. The probable subunits identified in analytical SDS-PAGE were found to be 71 & 74 kDa. Hence, the enzyme thus purified contained at least a single form of mGAD which is a heterodimer of 71 and 74 kDa; or two forms of mGAD, one a homodimer of 71 kDa and the other a homodimer of 74 kDa. These results suggest that the mGAD thus obtained is different from soluble GAD in its molecular structure (see Abstract in this volume, Bao, J. et al.), but similar to soluble GAD in its kinetic properties, as well as in its sensitivity towards heat treatment and various GAD inhibitors like AOA, PCMB and 3-MA. The physiological role of mGAD in the brain remains to be determined.

**581.5** MDMA AND PCA INCREASE 5-HT LEVELS EXTRACELLULARLY IN CULTURED RAPHE CELLS GROWN IN SERUM FREE MEDIA: POTENTIATED BY DEPRENYL AND DEPOLARIZATION AND ATTENUATED BY RESEQUELM, X.E. Guo and E.C. Armita. Dept. of Biology, New York University, Washington Square East, New York, N.Y. 10003

A novel serum-free microculture system coupled with HPLC-EC was developed in order to investigate the direct effects of 3,4-methylenedioxymethamphetamine (MDMA) and p-chloroamphetamine (PCA) on fetal raphe neurons. MDMA (10$\mu$M) and PCA (10$\mu$M) increased media 5-HT (20-40 nmol/mg; PCA > MDMA) as compared to non-detectable levels of 5-HT levels in untreated cultures. After 48 hours of incubation with MDMA, 5-HT levels increased in the media as much as 400% compared to the control cultures. Extracellular storage of 5-HT was diminished by renerin (5$\mu$M) and the MDMA-induced increase in media 5-HT levels was attenuated by 17% and 37% respectively. Furthermore, MDMA and PCA-induced release of 5-HT could be blocked by fluoxetine at 5$\mu$M. The MDMA-induced release appears to be both Ca$^{2+}$ dependent and -independent, even further attenuated by nimodipine at 100 $\mu$mol/L (a L-Type Ca$^{2+}$-channel blocker). Supported by NIDA contract# 271-90-7403.

**581.2** PURIFICATION AND CHARACTERIZATION OF SOLUBLE GLUTAMATE DECARBOXYLASE FROM PORCINE BRAIN. J. Baet, T.B. Nathan, and J.-Y. Wu. Dept. of Physiology & Cell Biology, Univ. of Kansas, Lawrence, KS 66045-2106

Soluble L-glutamate decarboxylase (GAD) was isolated from porcine brain by homogenizing the brain tissue in water at 4°C. GAD was first purified by DEAE-cellulose and hydroxyapatite chromatography. Two preparative PAGE activity were obtained on hydroxylapatite column. The major activity peak, referred to as soluble GAD (sGAD), was further purified by a combination of gel filtration column, preparative non-denaturating 7.5% and 5-25% gradient polyacrylamide gel electrophoresis (PAGE). This preparation showed a single protein band on non-denaturating 5-25% gradient PAGE, which coincided with GAD activity. The molecular weight of native sGAD was calculated to be 125.2±1.0 kDa from the non-denaturating gradient PAGE and 131±1.0 kD from Sephadex G-200 column. The purified sGAD preparation was dissociated into two protein bands of 64±1.0 kD and 59±2.1 kD on SDS-PAGE, suggesting that sGAD is either a heterodimer composed of either 64 kD or 59 kD subunits. Polyclonal antibodies were produced in rabbits by immunizing rabbits with ~150 μg purified sGAD preparation. The specificity of anti-GAD serum was established by immuno diffusion, immuno precipitation and Western blotting. Similar studies for the other forms of soluble GAD are in progress. (Supported by grants NS 20978 (NIH) and BNS-8820858 (NSF)).

**581.4** THE GLUTAMINE CONTENT OF ASTROCYTES: REGULATION BY pH, CAMP AND HYPERCORTISONE. N. Brooks*. Dept. of Pharmacol. & Expil. Therap., Univ. of Maryland School of Medicine, Baltimore, MD 21201

A change in extracellular pH from 7.4 to 7.8 caused a ~3-fold increase in the free glutamine content (Gln) of mouse cerebral astrocytes that were incubated with glutamine (Gln) and NHE-$^+$ (Brooks, J. Neurochem., 1992, in press). This effect of pH does not appear to result from increased free NHE$_2^+$ concentration or from changes in transport of Gln or Glu. Does the mechanism involve regulation of glutamine synthetase (GS) activity by pH? To examine the effect of induction of GS activity on Gln and glutamine levels of cell, the nonequilibrium cultures were pretreated with dibutyryl CAMP (dibAMP, 0.25 mM in serum-free MEM, changed twice during 6 h) or with hydrocortisone (HC, 1 μM in MEM supplemented with 5% fetal calf serum for 2 h). The cells were then preincubated to deplete them of free amino acids (1 h at 35°C in HEPES-Tween-buffered salts solution (HTB), pH 7.4), and then incubated for 30 min with 0.1 mM Glu and 0.1 mM NHE$_2^+$ in HTB at pH 7.4 or 7.8. Values of Gln, measured by reversed phase HPLC with precolumn deamination) were as follows:

<table>
<thead>
<tr>
<th>Condition</th>
<th>pH 7.4</th>
<th>pH 7.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unreated</td>
<td>15.5 ± 1.1</td>
<td>54.4 ± 4.3 (6)</td>
</tr>
<tr>
<td>dCAMP-treated</td>
<td>42.2 ± 14.5</td>
<td>104.1 ± 19.3 (3)</td>
</tr>
<tr>
<td>HC-treated</td>
<td>77.8 ± 37</td>
<td>228.2 ± 38.8 (3)</td>
</tr>
</tbody>
</table>

Clearance of Gln from the solution was 26-39% at 30 min. The results show that induction of GS activity markedly increased Gln, but that the pH effect remained large. This effect of pH may underlie the increased level of brain glutamine observed in hyperammonemia. (Supported by USPHS grant ES09392).


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Previous studies have shown that 3,4-methylenedioxymethamphetamine (MDMA) or ephedrine inhibits [3H]-SHT independent release of [3H]-SHT. These actions of MDMA are mediated by the serotonergic high affinity uptake site. It has been suggested that the mechanism by which MDMA releases 5-HT is via the exchange diffusion mechanism, i.e. MDMA is exchanged for SHT in the cyttoplasmic pool. Thus, MDMA is accumulated comparable to which 5-HT is released.

Our present study characterizes the accumulation of [3H]-(+)-MDMA (0.558 0f monoamine into rat brain synaptosomes (P2) and astrocytes). 3,4-Methylenedioxymethamphetamine (0.2-20mM) for the accumulation of [3H]-(+)-MDMA into synaptosomes indicated an apparent Km of 34m and a Vmax of 25pmol/mg protein/30min. Sonication produced a 40% decrease in [3H]-(+)-MDMA accumulation, while [3H]-SHT uptake was reduced by 70%. The accumulation of Sum of [3H]-(+)-MDMA was not significantly decreased by 100mM ouabain, while [3H]-SHT uptake was diminished by 70%. Long-term degeneration of serotonergic terminals with a dose of 10mg/kg p-chloroamphetamine (PCA) did not alter the accumulation of Sum of [3H]-(+)-MDMA, while it decreased the [3H]-SHT uptake by 40%. This result suggests that [3H]-(+)-MDMA is accumulated into rat brain astrocytes.

Our results indicate that [3H]-(+)-MDMA is accumulated into rat brain astrocytes. Saturation analysis of [3H]-(+)-MDMA accumulation into astrocytes exhibited an apparent Km of 0.3mM and a Vmax of 25pmol/mg protein/30min, with regional specificity. (Research Supported by NIDA contract# 271-90-7403).
581.7
SELECTIVITY OF VARIOUS SUBSTITUTED AMPHETAMINES
FOR MAO-A, E.T. KOKOTOS LEONARDI* P.X. HOU AND E.C. AZMITIA.
Dept. of Biology, New York University, N.Y. 10003.
PCAFFEN AND MDMA have been linked to serotonin neuropathy. We have
previously shown that increased MAO-A activity in culture. MAO-A
contains serotonin with a higher affinity than does MAO-B; serotonin-containing
cells contain mostly MAO-B. Dopamine is metabolized with equal affinity by both
MAO subtypes, and PCAFFEN is selective for MAO-A. Microanalytical analysis
was performed on the brain stem and hippocampal rat brain homogenates.
Selectivity for MAO-B was determined by pre-treating homogenates with 100 
NI-monoamine oxidase B inhibitor for 5 min. 37°C. Assays were performed at a final
concentration of 10 M-10 M, 10 mins, 37°C. 

We found no significant effects by any of the amphetamines tested on
MAO-A activity. MAO-A was significantly increased in control, 1.5 uM
with uM, 25 uM and >100uM respectively. In cultured serotonin
cells treated with 10 uM MDMA, the development of the uptake
was inhibited by 45% (p<0.001). This MDMA inhibitory effect was potentiated in
cultures that were treated with clorglyine (10um-100um) (60% inhibition, p<0.01).
These results suggest that inhibition of MAO-A may contribute to the
neurotoxic properties of these drugs. This work was supported by NIDA
contract #271-87-8144.

581.9
CHRONIC COCAINE AND HALOPERIDOL ELEVATE 3-METHOXYXYTAMINE
Neuropsychiatry Branch, NIMH Neuroendocrinology Center, St. Elizabeths, Washington, D.C. 20032, U.S.A.
According to previous reports, changes in tuberoinfundibular dopamine neuron (TIDA) activity do not correlate with the anterior
pituitary dopamine (DAM) content. Since the TIDA neurons do not form classical synapses and are juxtaposed to blood vessels, it is also
unclear whether 3-methoxyxynamine (3MT); a DA metabolite used as an
indirect index for DA release) can reflect activity of these neurons.
We measured 3MT in the anterior pituitary of male rats using gas
chromatography/mass spectrometry with negative chemical ionization.
The animals were treated chronically or acutely with 0.4, 
mg/kg of haloperidol (HAL) (3 days of weekly ip injections of HAL
followed by HAL or vehicle (VEH) injection 1 hour before sacrifice, or
3 weeks of daily ip VEH injections followed by HAL or VEH injection 1 hr before sacrifice), or
cocaine (10 mg/kg twice daily for 1 week followed by
1 week withdrawal versus twice daily injections of VEH
followed by 1 week withdrawal). An injection of monoamine oxidase inhibitor pargyline (75 mg/kg, ip) was given to the rats 10, 20
or 30 min before sacrifice by microwave brain irradiation.

Increased 3MT accumulation (133-142% of control, p=0.001) was
observed in the rats treated chronically and/or acutely with HAL.

Chronic cocaine treatment significantly elevated steady-state 3MT
levels (140% of control, p=0.05) and tended to increase 3MT rate of
formation (160% of control). These findings indicate that anterior
pituitary 3MT - in contrast to DA - may be a useful index of TIDA
neuronal activity.

581.11
Coexistence of vasopressin and oxytocin in rat magnocellular neurons upon
intrahypothalamic injections with vasopressin RNA. G.F. Jirkovski, D. Magierski-Leonor, F.E. Bloom.
Dept. of Neuropharmacology, Scripps Research Institute, La Jolla CA 92037
In previous studies we demonstrated that magnocellular hypothalamic
neurons in the homoyzognous Brattleboro rat are capable of accumulating,
transporting and translating exogenous vasopressin (VP) RNA, resulting in a
temporary correction of diabetes insipidus. In the present study intact adult
Wistar rats were injected intrahypothalamic with 32P-labeled VP RNA. The rats
were decapitated and the hypothalamus neurohypophyssial tract with [32P]-labeled VP RNA. After a survival time of 18h, radiolabelling could be observed in most of the VP immunostained
neurons. In addition, the distribution of the radioactivity was similar as well as in the
posterior lobe and labeled, indicating that VP RNA was taken up and transported through the tract as well as mono pyrined RNA. PCR-MAO Immunocytochemistry of consecutive semithin hypothalamus sections
revealed that in RNA injected animals 15% of the OT immunoreactive
perikarya contained this signal. This indicates that OT neurons can be recruited into
translation of exogenous VP RNA. In preliminary experiments, RNA
binding sites were localized on cryostat sections of rats hypothalami
by autoradiography. Elevated VP RNA is intensely
bound by fiber tracts, but was absent in the magnocellular nuclei. The present
findings indicate that uptake, transport and translation of VP RNA by
magnocellular hypothalamic neurons is not restricted to the
gene deficient Brattleboro rat but may represent a feature of the
hypothalamic neurons generally. Exchange of RNAs between neurons
could allow for the transitory coexistence of otherwise unrelated
neuropeptides.

581.8
EXPRESSION OF CATECHOL-O-METHYLTRANSFERASE (COMT)
Dept. Anatomy, University of Helsinki, Orion Pharmaceuticals, Espoo, and National Public Health
Institute, Helsinki, Finland.
Antisera against rat recombinant COMT were
raised in guinea pigs to reveal the distribution of
COMT in rat tissues. Immunoprecipitation of in
ultra-sensitivity and soluble COMT proteins indicated that both forms were effec-
tively recognized by the antisera. Western blotting analysis showed that both forms were detected in
rat kidney, liver, brain, stomach and adrenal.

Indirect immunofluorescence technique showed specific immunoactivity in the ependymal cells
lining cerebral ventricles, plexus choroides, median eminence, Bergmann glia of the cerebellum,
and pituicytes of the posterior pituitary. Cells lining the intermediate lobe were intensely
immunoactive. Hepatocytes, epithelial cells in the stomach and duodenum, muscle layer of the
gut, islet cells of the pancreas, and kidney tubules were also immunoactive. Preadsorption of
the antisera with recombinant rat COMT protein abolished all staining, and preimmune serum did
not stain any structures.

The results suggest that COMT is a widespread enzyme both in the brain and peripheral organs.

581.10
Uptake and stimulus-dependent release of [32P]-labelled Vasopressin RNA by hypothalamic primary cell cultures.
D. Magierski-Leonor, F.S. Jirkovski, F.E. Bloom
Dept. of Neuropharmacology, Scripps Research Institute, La Jolla CA 92037
We recently showed that magnocellular hypothalamic neurons in
Brattleboro rat can accumulate, transport and translate
exogenous vasopressin (VP) mRNA. To investigate the
mechanisms of RNA uptake, transport, and secretion, we
incubated primary cultures of dissociated fetal (ED 17) rat
hypothalamus with [32P]-labelled VP mRNA. Northern blots revealed that a distinct portion of cells, mostly neurons,
accumulate RNA in perikarya and processes. This group of
cells includes VP immunoreactive perikarya but is not limited to this
cell type. We could further demonstrate that depolarization of the
cells with 50 mM KCl increased the amount of radioactivity
released into the culture medium, indicating a stimulus
dependent secretion of VP RNA. This effect was in part Ca++
dependent. Stimulation of the cultures with 10-6 M of either
synthetic Ang-vasopressin or oxytocin resulted in release of
incorporated exogenous VP RNA, but 1mM glutamate did not
facilitate any release. Our results indicate that a stimulus
dependent secretion of certain RNAs might be a common
physiological feature of hypothalamic neurons, thus providing for
a novel way of interneuronal communication.

581.12
The PC12 cell line is derived from a rat pheochromocytoma. PC12 cells
grow better at high cell densities rather than low cell densities and under
these conditions metabolize glucose quickly. We examined the effect of
plating density and acute or sustained glucose depletion of <90% of initial
medium level on the growth rate, viability, media levels of lactate and glucose,
and dopamine storage in these cells.
Sustained (-5 days), but not acute (+1 day), glucose depletion decreased the
growth rate of PC12 cells. Unexpectedly, the viability, as well as in the
posterior lobe and labeled, indicating that VP RNA was taken up and transported through the tract as well as mono pyrined RNA. PCR-MAO Immunocytochemistry of consecutive semithin hypothalamus sections
revealed that in RNA injected animals 15% of the OT immunoreactive
perikarya contained this signal. This indicates that OT neurons can be recruited into
translation of exogenous VP RNA. In preliminary experiments, RNA
binding sites were localized on cryostat sections of rats hypothalami
by autoradiography. Elevated VP RNA is intensely
bound by fiber tracts, but was absent in the magnocellular nuclei. The present
findings indicate that uptake, transport and translation of VP RNA by
magnocellular hypothalamic neurons is not restricted to the
gene deficient Brattleboro rat but may represent a feature of the
hypothalamic neurons generally. Exchange of RNAs between neurons
could allow for the transitory coexistence of otherwise unrelated
neuropeptides.
Sustained (>5 days), but not acute (<1 day), glucose depletion decreased the
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findings indicate that uptake, transport and translation of VP RNA by
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gene deficient Brattleboro rat but may represent a feature of the
hypothalamic neurons generally. Exchange of RNAs between neurons
could allow for the transitory coexistence of otherwise unrelated
neuropeptides.

DIFFUSIONAL PROPERTIES OF DOPAMINE IN A RAT MODEL OF PARKINSON'S DISEASE AS MEASURED BY MULTIPLE IN VIVO ELECTROCHEMICAL METHODS. H. G. van Herik, J. Hobbie, R. J. Hoffman, and G.A. Gerhardt. Departments of Pharmacology and Psychiatry and The Rocky Mountain Center for Sensor Technology, University of Colorado Health Science Center, Denver, Colorado 80262.

Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra with a subsequent depletion of dopamine (DA) and DA nerve terminals, which contain a high-affinity DA uptake and vesicular DA storage site. Although current treatments include DA replacement strategies, the fate of DA in the extracellular space is poorly understood. The goal of this study was to determine the temporal and spatial diffusional properties of locally applied DA in the 6-hydroxydopamine (6-OHDA)-lesioned striatum as compared to the intact non-lesioned striatum and two control brain regions, the cerebral and cerebellar cortices, which are naturally devoid of DAergic nerve terminals. Male rats were unilaterally lesioned with 6-OHDA to remove the nigrostriatal DA-ergic pathway. Rats with greater than 80% loss of dopaminergic cells were determined by dopamine-BUDETHAL uptake. Histological confirmation and behavioral data confirmed this procedure. The data showed that the uptake of exogenously applied DA to the intact non-lesioned striatum and two control brain regions, the cerebral and cerebellar cortices, which are naturally devoid of DAergic nerve terminals, was significantly lower than that of the lesioned striatum. These results are consistent with previous studies.

581.14

OVEREXPRESSION OF GAP-43 IN A172 CELLS. C. Gamby, R. G. Allen, and L. Baizer, R. S. Dow Neurological Sciences Institute, Good Samaritan Hospital and Medical Center and C.R.O.E.T., Oregon Health Sciences University, Portland, Oregon 97239.

GAP-43 is a membrane bound phosphoprotein expressed primarily in neurons. While its precise function remains to be determined, it is concentrated in the growth cone area where it may be involved in the membrane addition associated with axonal growth. GAP-43 may also play a role in neurotransmitter and hormone release by facilitating the fusion of secretory vesicles with the plasma membrane. We have recently investigated the role of GAP-43 in secretion of adenocorticotrophic hormone (ACTH) by a pituitary-derived neuroendocrine cell line (A172). Western blot analysis demonstrates high levels of GAP-43 expression in A172/D16-16 cells. Rat or chicken cDNAs for GAP-43 under the control of the A172 promoter were transfected into A172/D16-16 cells and expression of exogenous GAP-43 was tested by RNAse protection and Western blot analyses. Cells overexpressing both rat and chicken GAP-43 have been isolated; none exhibit significant changes in cellular morphology. Spontaneous release of ACTH (measured by radioimmunoassay) by the clones overexpressing GAP-43 is similar to that in control cells, but the CRF-stimulated release is reduced by 30 % to 40 %. Supported by NIH NS26806.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS: ANATOMY

582.1


In vivo cat primary somatosensory cortex classically contains four cytoarchitectural subdivisions: area 3a, 3b, 1, and 2 (Staller and Mahr-Clement, '84) and each band appears to be functionally distinct. Anatomical and electrophysiological mapping techniques used to correlate somatotopic and functional organization within more than one band revealed important idiosyncrasies in size and distribution of pyramid cells within layer V of area 3b as observed from Nissl-stained material. In most cases there appeared to be significant changes along the lateral-to-medial axis in the number and size of large pyramidal cells, and with major body parts represented within area 3b. Layer V pyramidal cells located in the hindlimb representation appeared larger and more intensely stained than those observed in the forelimb representation. These cells are generally larger peripherally and interrupted. In some occasions, large pyramidal cells within layer V formed a continuous band spanning across all primary somatosensory cytoarchitectonic areas. However, in other cases, only a few cells were observed throughout the entire medio-lateral-to-lateral extent of the body representation within area 3b. Layer IV thickness was also found to vary within area 3b in the same animals. Confirmation of some cytoarchitectonic borders were obtained using ACHE histochemistry. The pattern of large pyramidal cells in layer V of cat primary somatosensory cortex appears to be an idiosyncratic feature and cannot be used alone as a factor for the determination of cytoarchitectonic borders. It has yet to be determined if these variations have any functional implications in the processing of sensory information. Supported by DGO9 fellowship to C.A. and MRC grant MA 8700.

582.3


The VPL, VPIL and CL in monkeys are a major source of input to the deep layer of the spinal thalamus, which has been proposed to have a role in conveying discriminative information about touch. In this study, we have examined the effects of cervical and thoracic spinal cord lesions on the input to spinal cord projects in monkeys. The results suggest that the VPL and VPIL are more effective in transmitting input to the spinal cord than the CL is.

582.4

DISTRIBUTION OF GABA A RECEPTORS IN CAT SOMATOSENSORY AND MOTOR CORTEX. J. Li and H.D. Schwark*. Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Rapidly-adapting (RA) and slowly-adapting (SA) submodality-specific regions have been described in area 3b of cat primary somatosensory (SI) cortex (Sretavan & Dykes, '83). Compared to SA regions, a higher proportion of neurons in RA regions are sensitive to tactile stimuli including bicusculine (Dykes et al., '84), suggesting differences in the organization of GABAergic systems between these two regions.

To determine whether GABA A receptor densities might correlate with the segregation of submodalities, we have examined the distribution of [1H]picrotoxin binding sites in film autoradiographs of parasagittal sections through SI. Reliability of patterns was determined in series of 4-6 closely spaced sections. Density histograms were used to measure the distribution of [1H]muscimol binding along the lateral vertical extent of cortical columns in areas 2, 3a, and 4.

Although initial visual inspection of muscimol binding in area 3b failed to reveal density differences related to submodality segregation, the issue is important because quantification of receptors with autoradiography is the only method available. We found that in area 3b, muscimol binding density was highest in areas 2 and 3a, and lowest in area 4. In areas 2 and 3a, the highest levels were in layers I and II (average values 1700-3200 pmol/mg tissue). Below layer II, levels of binding decreased with depth, except that in the most dense columns histograms, the upper layer IV showed a peak in binding density. Supported by NIH grant NS25729.
THE ORGANIZATION OF CORTICOFUSSOCAL AND CALLOSAL PROJECTION NEURONS AND CALBINDIN-IMMUNOREACTIVE NEURONAL POPULATIONS IN THE SECOND SOMATOSENSORY CORTEX. K.A. Bakker*1, P. Hermon and H.T. Chang. Dept. of Anatomy and Neurobiology. The Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163

We investigated the spatial distribution and collateralization of corticofugal and callosal projection neurons in the rat second somatosensory cortex (SII) and investigated whether these projection neurons were immunoreactive for calbindin. In double-labeling studies, retrograde tracers were injected into electrophysiologically-identified somatosensory areas of ipsilateral primary somatosensory cortex (SI) and motor cortex (M). calbindin-containing neurons were found in the lateral SI-superior parietal area with the SII somatotopic map. Injections into homotopic areas of SII and MI, or SI of both hemispheres in some animals, the SII representation area was mapped to determine the congruence of retrogradely labeled corticofugal and callosal neurons in the lateral SI-superior parietal area with the SII somatotopic map. injections into homotopic areas of SII and MI, or SI of both hemispheres in some animals, the SII representation area was mapped to determine the congruence of retrogradely labeled corticofugal and callosal neurons in the lateral SI-superior parietal area with the SII somatotopic map.
582.11 AXOSOMATIC SYNAPSES ONTO INTRINSICALLY BURSTING NEURONS IN RAT SI (BARREL) CORTEX.
E.L. WHITE, Y. AMITAI and M.J. GUTNICK, FACULTY OF HEALTH SCIENCES, UNIVERSITY OF SABRETHA, ISRAEL.

Recording under threshold conditions for synchronization shows that most regular spiking cells have strong and dominant inhibitory inputs, while intrinsically bursting cells show clear evidence of inhibition (Chagnac-Amitai and Connors, 1989). This study is part of a broader effort to determine if differences in inhibitory responses are reflected in the numbers or in the spatial distribution of symmetrical, presumed inhibitory synapses. Our approach is to use intracellular recording to classify neurons by their intrinsic firing properties, and to label them using HRP or biocytin. Then the neurons are processed for EM and serial thin sectioned. The somata and proximal dendrites are reconstructed and the distribution of their synapses displayed, using a newly developed system for capturing video images directly from the electron microscope and for making 3-D reconstructions from them. Two somata of intrinsic bursters were calculated to have surface areas of 541 μm² and 855 μm². These cells had 54 and 86 synapses respectively, that is, one synapse per 10 μm² of somatic surface area. The results of ongoing studies will determine whether this ratio of synapses to somatic area holds for other bursting neurons and whether it is shared by regular spiking neurons. NIH 19419, Israel Acad. of Sciences 2561/90.

582.12 MORPHOLOGY OF ELECTROPHYSIOLOGICALLY IDENTIFIED NEURONS IN ADULT RAT NEOCORTEX. E. Schedlitz* and H.U. Lohmann, Institute of Neurophysiology, University of Bochum, D-5800 Colenage, FRG.

We were interested in the morphological and electrophysiological properties of single supragranular cells in primary somatosensory cortex of mature rats. Intracellularly recorded neurons were selected on the basis of their characteristic properties and their synaptic inputs. After functional characterization neurons were labelled by intracellular injection of biocytin. Out of 85 stained cells, 33 neurons with a relatively complete axonal and dendritic arborization pattern were selected for detailed graphical reconstruction and subsequent morphometrical analysis. Resting membrane potential Vm was —81.5 ± 4.6 mV (mean ± SEM) and neuronal input resistance Rm was 38.9 ± 4.6 MΩ. All cells, including four slowly spiking neurons were classified as regular spiking cells with a prominent inhibitory synaptic input, consisting of a biphasic IPSP. Somatic area ranged from 96 to 744 μm² and did not correlate with Vm. The horizontal extent of the basal dendritic field ranged from 310 (mean ± SEM) microns. We used the methodology described recently by Chagnac-Amitai and Connors (1989). Supported by SFB 181/B4 and a grant from Ministerium für Wissenschaft und Forschung in NRW (H.U).


We studied the effects of histamine (HA) on unit activities in the somatosensory cortex of rats. Microelectrode penetrations and t-test of mean value of inter-spike intervals (MISIs) and normalized power spectral density function (PSDF) were used. We found that: 1) HA (0.5M) both raised (n=14) and reduced (n=6) MISIs in the recorded neurons (n=20), suggesting HA has both inhibitory and excitatory effects on the cortical neurons. 2) Cismedidine (0.1M) excited the neurons slightly and blocked the inhibitory action of HA (n=9), suggesting the inhibitory effect of HA is via the H2 receptor. Diphenhydramine (0.1M) inhibited neurons and did not block the inhibitory action of HA (n=9), suggesting the excitatory effect is via the H1 receptor. 3) HA reduced both the peak frequency and value of PSDF (p<0.01, n=20) and induced a 'post-inhibitory rebound' of the peak value with the peak frequency remaining low after iontophoresis, suggesting HA modulates the cortical rhythm and has after-effects.

583.2 SPINE STATISTICS AND RECEPTIVE FIELD PROPERTIES OF NEURONS IN PRIMARY SOMATOSENSORY CORTEX OF RAT. D. Yu-Zhen Lin, L. Yuan Liu, X. Pei-Xi Chen, S. Gang-Zhi, Y. Lawrence Bro. UC, San Francisco, CA, USA.

Neurons within the barrel field of rat SI cortex encode features of the deflection of a vibrissa (Simons, J. Neurophys., 1978). Here we report on the variability of this code in response to multiple presentations of a stimulus. Our measurements consisted of unit recordings from adult Sprague-Dawley rats anesthetized with urethane. Each stimulus trial consisted of displacing a vibrissa from its natural position. Vibration (-0.1 to 0.1 Hz) and angular velocity (-50°/s to 500°/s). Stimuli, chosen at random from the set, were presented at 1 s intervals and the entire set was presented up to 200 times. We observed: The mean number, ρ, of spikes elicited in units at the depth of layer 4 was relatively low, ρ < 2 spikes per trial, even for apparently optimal stimuli. The trial-to-trial variance in the number of spikes, σ², was equal or significantly less than its mean, i.e. σ² < μ (Fig. 2). Units at the depth of layer 2/3 fired multiple spikes per trial and exhibited a large, nonstationary variance. Units with similar orientation preference appear to be spatially clustered within a barrel.

The low variance in layer 4 units is reminiscent of that found in peripheral pathways and, notably, cortices used for pattern recognition (e.g., 2-4 μA, for units in cat and monkey VI cortex (e.g., Tolhurst et al., Vision Res. 20, 1989). The implication of the above observations for population coding is under investigation.


We studied the temporal transfer characteristics (TTC) of cortical neurons recorded in the barrel representation of SI (Barrel) of urethane-anesthetized rats. Tactile stimuli (TS) of 8 ms duration were applied by use of a computer-controlled mechanical stimulator as small skin indentations. Neuron responses were quantitatively analysed based of PSTHs. We varied systematically interstimulus intervals (ISI) between 20 and 200 ms and the number of stimuli (NS) in each train (2 to 6). Between each train was a pause of 3 sec. By this we were able to investigate the transition from transient to steady state conditions. In the double-click condition, i.e. using two stimuli, we found the often described TTC with low cut offs at ISIs between 20 and 80 ms. However, this TTC was not replicated when the ISI effect to the third or higher TS was tested. Accordingly, the overall dependencies depended not only on ISI, but in addition on the number of the stimulus and its ISI condition. Further analysis revealed that amplitude measures of peak height yielded only partial insight. Inhibition seen for short ISIs appeared to be uncorrelated with the preceding response. This leads to the observation that inhibitory action is restricted to given numbers of TS revealing strong sequential effects. The results are discussed in respect to modulation of neuron responses under natural conditions that are characterized by severe temporal constraints.

Supported by the DFG.

583.4 LAMINAR ANALYSIS OF THALAMOCORTICAL INTERACTIONS IN SOMATOSENSORY SYSTEM OF RATS. K.D. Alloway*, M.J. Johnson, and N.B. Wallace, Dept. of Neurosciences & Anatomy, M.S. Hershey Medical Center, Penn State University, Hershey, PA.

Thalamocortical neurons terminate within cortical layers IIb and IV where they synapse upon the soma and proximal dendrites of local neurons, and distal dendrites extending from neurons located in supra- or infragranular cortical layers. In view of differences in thalamocortical inputs to cortical layers and in thalamic connections, this study used electrophysiological techniques to detect a laminar sequence of sensory activation. Single neuron responses to cutaneous receptive field stimulation were simultaneously recorded in the ventrobasal thalamus and somatosensory cortex of halothane- anesthetized rats. Hairy and glabrous skin REs were activated by either air puffs or mechanical indentations, respectively. Response magnitude and latency analyses clearly indicate that the cortical inputs to thalamocortical neurons in layers IIIb and IV. Response magnitude is first highest in layers II, III, and lowest in layers IV, V, VI then in layers II, III. Cross-correlation analyses of laminar differences in thalamocortical connection strength are currently in progress. These preliminary results provide evidence in support of activity proceeding along serial and parallel circuits within a cortical column. Supported by NIH grant NS-25363 and PSF RIO-032-22.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
SOMATOSENSORY REHABILITATION reciprocal responsive or focusing from Sch.

SOMATOSENSORY ENABLED movements, with NAD+ and halothane, was stable for many hours. On-line spike waveform analysis, perievent time histograms, and cross-correlograms guided identification of related cell pairs. Iontophoresis onto cortical neurons and controlled whisker stimulation enabled characterization of the transmitters used by physiologically connected cells. Several cell pairs, with and without shared whisker inputs, had correlated activity. These studies show whether cortical neurons receive common or direct interbarrel synaptic connections and which transmitters mediate these interactions. (Supported by NINDS 2012)

CONNECTIONS

The somatosensory region (SIV) of the anterior ectosylvian sulcus (AES) is an important source of inputs to the superior colliculus (SC) a structure involved in orientational and localization behaviors. SIV also shares reciprocal connections with somatosensory cortical regions, SI and SII, involved with tactile perception and discrimination. However, it is not known whether the properties of neurons are similar to those in S1 and SII or to those in the SC. Therefore, this investigation compared the receptive properties of SIV neurons to those of the SC, S1 and SII.

The receptive fields and responses of SIV somatosensory neurons (n=23) in cats (n=3) were examined. All neurons were rapidly-adapting and activated by low threshold mechanoreceptors. While the majority required high velocity stimulation and were broadly tuned to different directions of stimuli, few exhibited directional selectivity or spatial summation, and none showed spatial inhibition. Most exhibited medium to large receptive fields that contained areas ('hot spots') where a significantly higher response could be elicited than elsewhere in the receptive field.

The receptive field size, rapidly-adapting nature, and velocity sensitivity of SIV neurons are features similar to those observed in the SC or S1 or SII.

Supported by BNS 8719234 and EY 05554.

DISTRIBUTION OF PROPRIOSPCEPTIVE INPUT TO PERICIRCUIATE CORTEX OF AWAKE CATS. Jefferson C. Stimpson* and Arnold L. Tong, Dept. of Rehabilitation Medicine and Dept of Physiology and Biophysics, Univ. of Wash.

A previous report (Stimpson 1978:76) concluded that the data from three restricted but well sampled regions of pericruciate cortex of awake cats failed to support any model of topographic organization that emphasized the bounded regions within which all neuron response properties are the same. The data reported here, taken from a larger extent of pericruciate cortex in four cats, confirms this conclusion. Focusing only on responses to the visually evoked cutaneous neurons, most tracks contained a mixture of modalities: joint, muscle/tendon/joint, and active (during movement but not drinkable). Many neurons responded to movement or two or more joints. About half of the neurons recorded simultaneously or sequentially showed reciprocal sensitivities (e.g., flexion/extension or adduction/adduction). However, given a neuron with one pattern of response, the probability that the next neuron would show that same pattern was equal to that expected by chance, i.e. the distribution of input was random. Tracks in which many elbow-sensitive and wrist-sensitive neurons were found yielded similar results, although there was a slight tendency for elbow and wrist neurons to clamp. Neurons responsive to movement of two joints showed no preferred relationships, e.g. there were equal numbers of elbow flexion/wrist extension as elbow extension/wrist flexion, etc. These findings suggest that restricted areas of cortical cortex receive input from more diverse and scattered sources than had previously been thought. Even somatosomy was rather more fuzzy than precise. Neighboring neurons with reciprocal response patterns behaved as though they mutually inhibit one another; they could serve to code joint positions through their differential action. (This work was supported by USPHS Grants NS00396 and NS035082.)

RESPONSES OF CAT SI S1 RAPIDLY ADAPTING NEURONS TO CONTROLLED MECHANICAL STIMULATION. H. Estes*, M. J. Pettit and H. D. Schwark, Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Cutaneous rapidly-adapting first order fibers of many mammals have been classified by their response to the rapidly displacement components of controlled stimuli (Burgess and Perl, 1973). We have used constant-velocity ramp stimuli and a modification of the Burgess and Perl criteria (1973) to study single neurons in the primary somatosensory cortex of unanesthetized cats.

Thirty-five of 140 units responded well to single probe stimuli and were analyzed in detail. G1/F2 units (9/35) responded to the highest ramp velocities; G1/F2 units (9/35) showed intermediate properties: an initial brief response was followed, after a period of low or no activity, by a response during the remainder of the ramp. Five units had responses which were combinations of these patterns, suggesting convergence between groups.

The instantaneous firing frequency of all units generally increased with increasing ramp velocities. The average firing frequency of G1/F2 and G1/F2 units also increased in a graded manner with ramp velocity, whereas the response of G1/F2 units was discontinuous. These results suggest that rapidly-adapting cells in SI cortex which respond to punctate stimuli have response patterns similar to those of first order afferents. Supported by NIH grant NS25729.

OPTIC NERVE EXCITATION OF PERICIRCUIATE CORTEX IN CAT. A.L. Tow*

Single neurons were recorded on both sides of the cruciate sulci in chloralose-anesthetized cats. About 24% of the cutaneous small-field neurons and all of the wide-field neurons were excited by input over each optic tract. In addition, about 74% of the neurons failed to respond to skin stimuli, but were excited by optic chiasm input. Response latencies to optic chiasm stimulation fell into four distinct groups. Timing relations suggested the first surge arrived via superior colliculus and the third via visual cortex; the motes for the second and fourth surges remain unidentified. This was no indication that these separate surges converged onto the same neurons. Input via the calcarine optic tract evoked activity at a higher threshold, produced fewer spikes per discharge, and has a longer latency than did the ipsilateral optic tract. The difference in response latencies was largest for neurons responding in the first surge, and decreased progressively through the later surges. The mean latency of response to optic chiasm input was the same as that to calcarine forepaw stimulation, though optic chiasm input evoked more spikes per response. It is suggested that the visual input to pericruciate cortex serves to modulate on-going cortical output and thereby modulates the behavior of the animal. (Supported by USPHS grant NS00596).

SCALP POTENTIAL TOPOGRAPHIES EVOKED BY SURFACE STIMULATION IN MAN. R. Dowman*, T. H. Dacey, Dept. Psychol., Clarkson University, Potsdam, N.Y. 13699.

The 12 mV epoch following the evoking stimulus could be separated into four stable periods (SP): i.e., consecutive time points where the patterns were the same. For each SP the onset and offset latencies and the topographic patterns were comparable across subjects. The topographies for each of the first five SPs (9-80 ms, 90-117 ms, 135-156 ms, 180-219 ms, and 225-275 ms, respectively) were stable across all stimulus levels, suggesting that the sources (neural generators) underlying a given SP were the same at noxious and innocuous levels. The first two SPs (0-35 ms) were stable across both levels but not innocuous levels, suggesting that the sources underlying SP6 are different at noxious vs. innocuous levels. This finding is an easy putting these data using dipole source modeling to test hypotheses about the location of the sources generating these potentials and to quantify the source amplitude and duration. This analysis will allow these putative sources behave similarly to neurons involved in innocuous and noxious somatosensory cortical processes identified in animal studies.

A high-resolution magnetoencephalography was used to determine the representation of snout in the cortical region of the juvenile swine (3-6 weeks old, 5-10 kg). Each of five locations on the snout was transcutaneously stimulated with a pair of concentric circular electrodes (0.3 ms, 6 A) attached to an isolated stimulator to produce activity in the cortical region of the swine anesthetized with ketamine (10 mg/kg, i.p.) and xylazine (4 mg/kg, i.p.). The somatosensory evoked field (MEF) associated with the neural activity was measured on a plane 1.2 mm above the apex of the intact dura mater with a 4-channel superconducting magnetometer with a passband of 0.3 Hz to 1 kHz, after removing the scalp and the dural half of the skull. The stimulation of each site produced an MEF detectable on single epochs. The topography of averaged MEF normal to the measurement plane indicated that the cortical tissue active at 15 and 19 ms after stimulation was located systematically around the contralateral coronal gyrus, confirming the electrophysiological work of Craner (Ph.D. Thesis, East Carolina Univ., 1988). The lateral, top, midline and bottom regions of the snout were located in the medial, posterior, lateral and anterior portions, respectively, of the snout area. Some areas of the snout were represented in two sulci on each side of the gyur. Tugenial field potential data, however, indicate the existence of a somatotopically organized projection of the snout in the coronal gyrus and in the surrounding sulci.

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584.3

The TRN is composed of a thin layer of GABA-ergic cells surrounding the lateral and posterior thalamus, and therefore is not a thalamic nucleus. Its function is not completely understood, but appears to integrate ascending information from the thalamus, several brainstem nuclei and corticalafferents, all inputs from the internal capsule. We have examined the input to the somatosensory cortex to determine the nature of the synaptic contacts formed in the TRN, and to describe the placement of those contacts in the TRN. To label the cortical inputs into TRN, we injected small amounts (<0.2 µl) of 4% WGA-HRP in the somatosensory cortex of Macaque monkeys.camatano, allowing the animals to survive 2-4 days, perfused with mixed aldehydes, and prepared tissue for electron microscopic visualization of the transported WGA-HRP.

Results of these experiments show that cortical projections to the TRN end as synaptic terminal profiles similar to those described for cortical projections to thalamic sensory nuclei. The terminals are small, contain round vesicles, and form asymmetric contacts with small caliber dendrites in the TRN. We have also observed in single section what appear to be dendro-dendritic contacts between TRN neurons. We conclude that GABAergic inputs to the TRN may be as important in the thalamus, some of which contact one another, to result in feed forward inhibitory mechanisms. Supported by NS-23347 and NS-21445.

584.5

This study examined the somatotopic organization and modality of inputs to the primary somatosensory areas in the somatosensory thalamus retrogradely labeled by injections of retrograde tracers into the forepaw representation area of the primary somatosensory area (SII). Conventional extracellular electrophysiological mapping techniques were utilized. Injections of retrograde tracers were made into SI before or after the somatosensory thalamus was electrophysiologically mapped.

Most retrogradely labeled neurons were located in the ventroposterior lateral nucleus (VPL), rostral and medial region of the posterior complex (POdM), and the central lateral nucleus of the intralaminar complex. We recorded the number of firing in VPL and POdM. Combined retrograde labeling and mapping results show that SII projecting neurons in VPL: 1) were overwhelmingly activated by low-threshold mechanical stimulation of small receptive fields on the glabrous skin of the forepaw, 2) had responses that slowly adapted to maintained stimulation, and 3) were somatotopically organized. That only labeled neurons in VPL were not driven as readily as those in VPL; however, when driven, they were typically activated by intermediate- to high-threshold stimulation.

We conclude that SII projecting thalamocortical neurons in VPL receive primarily low-threshold, slowly adapting inputs from the cutaneous surface of the forepaw. Given their rostral location within VPL, neurons that project to the SIDo area do not overlap with those that project to the SII forepaw representation area. (Supported by The Center for Neuroscience of The Univ. of Tennessee, Memphis).

584.8
EXCITATORY POSTSYNAPTIC POTENTIALS (EPSPs) EVOKED IN NUCLEUS RETICULARIS BY MINIMAL STIMULATION OF THALAMIC RELAY NUCLEI. S. Rapdour, J. Deuchars and A.M. Thompson (SPON: Brain Research Association) Dept. Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

Nucleus reticularis (RTN), the major source of inhibition in rat thalamus, receives major inputs from cortex via internal capsule (IC) and from thalamic relay neurons. This study compared EPSPs evoked from IC with those evoked by micro-stimulation of relay areas, recorded intracellularly in vitro from RTN neurons identified electrophysiologically and morphologically. Both types of EPSPs were fast (width at half amplitude 6.9±2.8, IC and 5.1±1.1 ms, relay) at around ±80mV. Although the peak of the EPSP often exhibited a conventional voltage relation, EPSPs increased in duration with depolarization (De Curtis et al., Proc. EPSs fluctuated in amplitude, the relay input dramatically, with a peak standard deviation of 20% of EPSP amplitude. Relay EPSs exhibited profound paired pulse facilitation, but declined in average amplitude with maintained stimulation at 1Hz. Both stimuli could evoke variable and long latency bursts of EPSs whose time course resembled those of evoked relay EPSs. These may result from activation of slow spikes and burst firing in pretypic relay neurons within the slice.


The thalamic reticular nucleus (RT) is thought to control sensory transmission through the single neuron recordings in the thalamic reticular nucleus (RT) and the ventral posterior (VP) thalamic nuclei. This study was performed to determine whether the RT neurons are mutually inhibitory. Computer models employing such competitive inhibition phenomena often exhibit stable, low-amplitude, nearly sinusoidal periodic activity. The aim of this study was to determine whether such state changes can be seen in the spatio-temporal patterns of activity in RT and VP neurons. Results: The RT and VP thalamic nuclei in awake and anesthetized rats. In the pentoobarbital anesthetized state (0-10 Hz), the spontaneous activity exhibited a prominent cross-correlation of the RT neurons recorded in both phase synchronization. Both types of patterns were often seen at different times. The spontaneous transitions between in and out of phase oscillations. These RT neurons also exhibited a distinctive series of responses to peripheral stimulation, including: (1) an initial excitation, lasting up to 60 ms post-stimulus, 2) a strong inhibition, lasting up to 120-150 ms post-stimulus, and 3) a series of 7-10 Hz oscillatory bursts, which persisted up to 2-3 sec after the stimulus. These burst were also some patterns of synchronization, but they are often switched on or off or out of phase pattern. The results of the stimulus frequency from 0.2 Hz to 1 Hz resulted in a 160° phase advance, in which mice neurons were suddenly began their bursting pattern about 60 ms earlier. To conclude, the RT-VP thalamic network does not show simple synchronization, but instead exhibits complex dynamical state changes. Since the RT-VP thalamic network is decelerated, the phenomenon is reminiscent of the sudden frequency bifurcations characteristic of non-linear dynamical systems, especially those containing coupled oscillators. Supported by grants NS23722, AFOGR-90-0266, and AA06965 to JKC.


Previous recordings in anesthetized animals have suggested that the thalamus contains a detailed somatotopic map of the cutaneous periphery formed by extremely fine receptive fields (RFs). However, very few data are available in awake animals to validate these projections. We approached these issues through the analysis of simultaneous records from the extracelllular activity of up to 24 simultaneously characterized single neurons per animal in awake adult rats. Overall, it seems that the ventral posterior (VP) thalamic nuclei were recorded in 10 adult hooded rats in which 6 to 16 100-200-µm microwires were chronically implanted. Sensory stimulation (5-10 degrees, 1 Hz, 100 ms) of several single facial whiskers per animal were produced by a computer-controlled probe. VP neurons responded vigorously to the stimulation of multiple whiskers at different latencies (mean 6 ms) defining extremely large RFs (covering circular areas of 2-5 whiskers in diameter). Stimulation of single whiskers produced evoked and late responses, and the temporal pattern of spike responses. Changes when different whiskers were stimulated. In particular, many neurons exhibited time-dependent spatial shifts of their RF centers over the 50 ms following the stimulation. We have observed changes in RF sizes, and the directionality of the responses depends on the size of the RFs. The results suggest that individual animals can represent different responses in which networks of neurons process somatic information through highly distributed time-dependent mapping functions. These may provide the thalamus considerable plastic potential if even in adult animals. Supported by grants NS23722, AFOGR-90-0266 AND AA06965 to JKC.

CIRCUIT OSCILLATIONS IN A NEURONAL NETWORK MODEL OF THE SOMATOSENSORY SYSTEM. J. P. Utr, and J. K. Chapin. Department of Physiology, Hahnemann University School of Medicine, Philadelphia, PA 19102-1162.

As a tool for investigating the mechanisms underlying spontaneous oscillations and sensory responses, we created a network model system in a computer model was developed. This model consists of four layers, each containing a 30x30 grid of elements. The layers are connected with a pattern which resembles, in a simplified fashion, the axonal connections between the ventral basilar thalami (VT), the nucleus reticularis thalami (RT), the thalamic cortical layers (CT), the VM, a layer of inhibitory interneurons (IN). Each "neuron" incorporates several biologically realistic features, including: 1) membrane potentials and conductances, excitatory potentials, and synaptic potentials for several ion currents (Na+, K+, Cl−), 2) a membrane leakage conductance, and 3) a substantial time scale for the voltage-dependent potassium conductance, 4) 4) a voltage-sensitive spike-triggering threshold, and 5) axonal conduction delays. State variables (e.g., conductances and VM) are recalculated every 20 ms. The model simulates a 30x30 grid of elements. The model simulates a 30x30 grid of elements. The resulting patterns of similar firing, and the spatial patterns of spaltemporal oscillations depending on the values of certain parameters. For example, oscillations may be further subdivided into: 1) increasing the strength and frequency of the excitatory feedback, 2) decreasing the strength of a medium duration afterhyperpolarization (mAH, N = 20), and 3) decreasing the input to feedback from the VP. Supported by grants NS23722, AA06965 and AFOGR-90-0266 to JKC.


In recent investigations we have routinely obtained simultaneous extracellular single unit recordings from 24 single neurons in the somatotopically organized thalamus and/or cortex system of awake, behaving cats. Here we describe the use of multivariate statistical techniques to characterize the information and population coding by single neurons. The technique of discriminant analysis (DA) was used to determine how well a population of simultaneously recorded neurons can discriminate between different behavioral states. Only 10-23 neurons recorded during repetitive sensory stimulation were used to generate a matrix of discriminant functions which were used to define a discriminant. Even though single neurons by themselves were found to be poor predictors of whether a certain stimulus had occurred, their discriminability improved greatly when increasing numbers of neurons were used for the discrimination. Furthermore, most of these neurons were found to contribute to several types of discriminative behavior. Principal components (PC) analysis was used to extract the factors which underly activity in neuronal populations. For this, the single multivariate sensory discharge recorded over a range of stimulus conditions was time integrated and used to generate a correlation matrix. This matrix was rotated to generate a series of principal components, i.e., weighted combinations of the original variables (neurons). Each of these reconstructed components was generally found to reveal, in a statistically improved fashion, definable factors underlying activity within the neuronal populations, e.g., different aspects of sensory responses, movements or network oscillations. Similar analyses performed on data obtained from computer models of neuronal networks verified that PCA yields a spectral decomposition of the activity within the network. This optimally summarizes the network's functional organization. In conclusion, these results provide evidence for a distributed representation of information within the recorded neuronal populations. Supported by grants NS23722, AFOGR-90-0266, and AA06965 to JKC.

SOMATOSENSORY STIMULATION SUSPENSES 8-12 Hz OSCILLATIONS IN THE VENTRAL POSTERIOR COMPLEX OF AWAKE RATS AS PREDICTED BY A COMPUTER MODEL OF A. L. Nickoloff, J. C. Chapin, Department of Physiology and Biophysics, Hahnemann University, Philadelphia, PA 19102.

Thalamic neurons exhibit oscillations which can be clearly correlated with different states of the sleep cycle. However, very little is known about the physiology of oscillations in the range of 8-12 Hz observed in awake animals. It has been shown that we have simultaneously recorded the extracellular activity of up to 24 statistically characterized single thalamic neurons per animal in each of 5 awake rats. Oscillations in the range of 8-12 Hz in the ventral posterior complex of awake rats which were not carrying out either active sensory or motor tasks. These oscillations, which resembled the sharp thalamic oscillations and the slow "somatosensory rhythm", were observed in cats, occurred upon cessation of whisker or paw movement. These oscillations could be promptly disrupted by cutaneous stimulation. Long recordings durations revealed that thalamic neurons dynamically switch from a tonic firing mode (around 7 Hz) to a bursting mode (8-12 Hz). Similar oscillatory behavior was observed in a model of the rat somatotopic system which contains multiple thalamic, thalamic, and cortical compartments but no individual pacemaker elements. In this model, background oscillations in the thalamus were produced by modulatory influences on the thalamus. These results suggest that in the behaving rat VP neurons dynamically switch from a tonic to a bursting mode and that this latter may be responsible for gating the flow of sensory information conveyed by the thalamus to cortex. Supported by grants NS23722, AFOGR-90-0266 and AA06965 to JKC.


During slow wave sleep, the NT synchronizes rhythmic thalamo-cortical activity and contributes to the generation of spindle waves. The ionic mechanisms of rhythmic firing in rNT cells were investigated with standard in vitro slice techniques.

We found that endogenous rhythmic bursting of rNT cells is mainly due to the interplay of low-threshold calcium-dependent spike (rNT) and Ca2+ activated K+ current (IKA). Ca2+ and IKA activated non-specific cation current (ICAN) according to the following scheme.

Maximal block of a leak potassium current by HST resulted in 30-60 Hz tonic firing owing to the inactivation of IKA and the activation of a persistent sodium current.
A NEW TOOL TO CHARACTERIZE NOURAL DISCHARGE: COUNTING STATISTICS OF \( f^+ \) FLUCTUATIONS. K.-D. KNIFFI, M.K.C. MENGEN and C. VARELA-HICIA. Physiological Institute, Universität Würzburg, Röntgenring 9, 67071 Würzburg, Germany.

Temporal fluctuations in natural phenomena in the absence of intentional stimulation are not always the consequence of statistically independent random events. It has been shown that temporal fluctuations found in phenomena as different as membrane currents, earthquakes, intensity of sunspots, heart beat or breathing activity can be characterized by their power spectrum density \( S(f) \). In all three different systems \( S(f) \) is decaying as \( f^+ \) at low frequencies with \( b \leq 1 \). This behaviour of \( S(f) \) is called \( f^+ \) noise. Usually \( S(f) \) is obtained by Fast Fourier Transformation (FFT). To avoid the well known problems of using FFT for neural spike trains, a new method (Menne, Grün, Flachenecker, Kniffl) is submitted to analyse the low frequency part of \( S(f) \) of the recorded action potentials.

The series of recorded action potentials is considered to be a point process described as \( y(t) = \delta(t-\tau) \), where \( \delta(t-\tau) \) represents Dirac’s delta function, and \( \tau \) is the time of occurrence of a particular action potential within the spike train. The entire observation time was divided into counting windows \( A \) and the variance of counts \( Var(N(A)) \) was determined. This was repeated for different lengths of \( A \). If \( Var(N(A)) = (\Delta A)^{b} \), the power spectral density \( S(f) \) scales as \( S(f) \sim f^{b} \) with \( b \leq 1 \). If \( \tau = 0 \) in thalamonic neurons tested so far, the variance-time curve \( Var(N(\Delta t)) \) was proportional to \( (\Delta t)^{b} \) with \( b \approx 0.5 \), thus indicating that the neuronal discharge exhibited \( f^+ \) fluctuations. The applied method reliably discriminates these fluctuations from a pure random process, in which case \( b = 0 \).

We speculate that the basic mechanisms underlying neuronal activity in thalamic sensory systems are expressions of a “self-organized critical state”, as introduced by Bak, Tang and Wiesenfeld (Phys. Rev. Lett. 59: 381, 1987).

VISUAL CORTEX: BRAIN IMAGING TECHNIQUES


We have previously shown that changes in venous blood oxygenation produce Blood Oxygenation Level Dependents (BOLD) changes in water proton magnetic resonance signals in mammalian brain (1-2). We demonstrate that the effect is produced by a magnetic susceptibility change in venous blood vessels produced by changes in the concentration of the paramagnetic molecule deoxyhemoglobin and suggest that BOLD contrast imaging could be used for human functional brain mapping. We have now demonstrated that visual stimulation produces an easily detectable (5-20%) transient increase in the intensity of water proton magnetic resonance signals in human primary visual cortex in gradient echo images obtained at 4T field strength. The accompanying figure shows the time course of change in signal intensity produced in a region of primary visual cortex by two periods of a patterned flash binocular visual stimulus. The change is consistent with a neuronal produced neural activity increasing an increase in venous blood oxygenation. With the present 4T imaging system, we can follow changes with a temporal resolution of ~3 sec and with 1.6 x 3.1 x 10 mm image voxel size. Images have been assembled into real-time movies that show the temporal and spatial changes.


Figures 1, 2, and 3 illustrate the capacity of human visual cortex to resolve the anatomy of human brain. Recently, Belliveau and colleagues (Science, 254: 621-678, 1991) have reported the use of echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity. The echo planar magnetic resonance imaging (EPI) to image patterns of neural activity.
585.5

AREA V5 OF HUMAN VISUAL CORTEX IDENTIFIED IN INDIVIDUALS USING Positron Emission Tomography: IMAGING.
D.C.Watson, S Shippe, G.P Prackowski* and S.Zeki. MRC Cyclotron Unit, Hammersmith Hospital, London W12 OHS and Dept of Anatomy, University College, London WC1E 6BT.

We have previously used positron emission tomography (PET) to find the visual motion centre (V5) by averaging groups of subjects. Now, we report findings from individual subjects scanned with a Siemens-CTI 935B PET camera, with its spatial resolution enhanced by removal of the collimating system, to allow collection of data in 3D mode. Images of regional cerebral blood flow (rCBF) were obtained by using intravenous infusions of H215O. 12 subjects viewed computer displays of moving or static checkerboard patterns. Each subject had 6 scans in each of the 2 conditions. Statistical parametric maps (SPMs) were then used to indicate areas of the brain in which significant rCBF changes took place in response to visual motion. We also obtained high resolution magnetic resonance imaging (MRI) scans for all subjects and then coregistered individual PET-SPM's onto the respective MR images. Thus we could relate the sites of individual statistically significant activations to specific gyri and sulci of the individual brains. SPMs revealed bilateral activation of the area previously defined as V5 in all subjects. It was located ventrolaterally in the anterior part of the occipital lobe. The rCBF increase in V5 ranged from 2.4% to 8.6%. On normalization to Talairach stereotactic co-ordinates the locations of the visual threshold for activation at V5 varied within a range of 24 mm for either hemisphere. In addition to V5, our stimuli activated sites in the cuneus and the lingual gyrus, and these may correspond with the superior and inferior divisions of monkey V2/V3.

585.6

PET STUDY OF STEREOSCOPIC. A. Pito*, R. Zatorre, M.
Petrides, S. Frey, B. Alivisatos and A. Evans. Montreal
Neur. Inst. McGill University, Montreal.

To map the areas of the brain involved in global stereopsis, regional cerebral blood flow was measured by means of positron emission tomography(H215O bolus technique) in nine right-handed male adults under three conditions of testing: 1) DEPTH: a random dot depth map was projected either to a vertical or a horizontal bar at 100% binocular correlation. 2) SHAPE WITHOUT DEPTH: a 2-D vertical or horizontal black outline of a bar drawn on a background of random dots. 3) NEUTRAL: random dots without form or depth. Increases in blood flow due to depth perception were assessed by subtracting activation in the shape condition from that in the depth condition. Results showed activation on the border of areas 17 and 18 in the right cerebral hemisphere and in areas 19 in the left cerebral hemisphere. When the neutral condition was subtracted from the depth condition a focus was obtained only in the right cerebral hemisphere again along the border of areas 17 and 18. These data are concordant with evidence of a right hemisphere superiority for stereopsis, and they indicate that the analysis and processing of unambiguous stereoscopic information high in binocular correlation begins early in posterior visual areas of that hemisphere.

585.7

STIMULUS SPECIFIC INCREASE OF OXIDATIVE METABOLISM IN VI-

The mechanism that mediates the coupling of neuronal activity to blood flow and oxidative metabolism have been the subject of controversy. Previous PET studies have demonstrated an apparent uncoupling of blood flow and oxygen metabolism in both somatosensory and visual cortex (Fox et al., 1988.) The reported changes in oxygen metabolism have been considerably smaller than those reported for blood flow and glucose consumption (25%-50%). Using a newly developed method for measuring oxygen consumption using a single bolus injection of [15O]O2 (Ohno et al., 1992) with 3 minutes of multi-frame data acquisition, we confirmed the lack of a coupling of blood flow to oxygen metabolism in somatosensory and visual cortex (Fujita et al., submitted). We hypothesized that the visual stimuli would be incapable of modulating oxidative metabolism in visual cortex, since primary V1 has a heterogeneous distribution of cytochrome oxidase (blots) that has been correlated with glucose uptake and stimulant variables in the macaque (Towell et al., 1989).

Measurements of oxygen metabolism and blood flow in normal volunteers were measured using separate bolus injections of [15O]O2 and [15O]O2 PET scans. Subjects had the right eye covered and raised a cross on a computer monitor with the left eye. During the activation scans a semi-annual reversing contrast stimulus was presented to the left visual field of the left eye at a retinal eccentricity of about 15°. Correlative MRI scans were obtained from all subjects. A focal increase of oxygen consumption (>30%) was measured in primary visual cortex. This result is consistent with a stimulus specific modulatory effect of oxidative metabolism in visual cortex.

586.1


In previous population studies (Zwier et al, Neurosci, 1989) to simulate laser effects on human visual performance, we utilized laser induced deficits obtained in rhese contrast sensitivity to alter the spatial frequency content of complex images used in human detection and recognition studies. In this paper five human laser accident cases are reported where the spatial characteristics of their visual defects have been characterized. Human laser ocular accident injuries suggest the presence of both absolute and relative areas of the visual field. A visual acuity loss of >2.0 diopters is a clinical measure of peripheral retinal fibrosis. Contrast sensitivity measurements reveal high as well as mid to low spatial frequency contrast sensitivity loss relative to non-exposed eyes. Visual threshold field loss may exceed 30 degrees where mid to low spatial frequency sensitivity loss is extensive. Retinal fibrosis, traction, and photoreceptor alteration are suspected in mediating the spatial vision degradation of the relative scotoma. Our results suggest that future perceptual simulations of such retinal injuries should incorporate the complex representation of both the absolute and relative spatial aspects of the induced scotoma into the simulated image.

586.2

RESIDUAL VISUAL FUNCTIONS IN THE BLIND FIELD OF A HEMIOPHIC PATIENT. C.M. Weis Erlmayer*, F. Fendrich and M.A. Gazzaniga. Center for Neurology, Univ of California, Davis CA 95616

Previously, we reported the existence of a small island of residual visual function in the blind left field of a hemianopic patient. CLT is a forced choice task, CLT reliably detects a 1 deg black stimulus flashed within this island. Above chance detection was obtained for central and vertical deficits. However, we report a patient with binocular hemianopic PLT and a vertical meridian. We now report results of additional testing which further characterize residual visual functions in this patient's blind field.

We performed spatial frequency mapping to the boundaries of the isolated island within the scotoma. The results of this testing suggest that the island of sparing is primarily confined to an area not more than 1 deg. We found PLT could not identify the location of stimuli presented within the island of sparing. He was also unable to direct a saccade toward 1 deg stimulus flashed at this location. Nevertheless, he could discriminate between a diamond and a square (both 1 deg on a side) centered within the island of sparing. However, when we increased the size of the diamond and square to 2 deg on a side, CLT performance decreased. The absolute size of the smaller stimulus was based on form or edge detection within the island of sparing. Larger stimuli completely blanked the island sparing the subject from this information.

We also investigated CLT's residual abilities at other locations within his blind field. Near the vertical meridian, CLT was able to direct a saccade toward flashed stimuli, but could not discriminate between a 1 deg diamond and square. On the other hand, he could discriminate between 2 deg diamonds and squares. This demonstrates a dissociation which is in direct contrast to that found at the island of sparing.

Supported by NIH/NINDS P01 NS17778-10 and the McDonnell-Pew Foundation.
586.3 DETECTION OF TEXTURE- AND MOTION-DEFINED BOUNDARIES BY NONRETRARDED AND MENTALLY RETARDED ADULTS. *Stephen Drosa III, Robert Fox.* Department of Psychology, Vanderbilt University, Nashville, Tennessee 37240.

Forms defined solely by relative differences in binocular disparity or motion have been difficult to detect for most mildly mentally retarded adults (MMR) to either discriminate between or to detect. Because both binocular disparity and motion are processed preattentively by early components of the visual system, we conclude that these findings is that the neural integrity of the visual system is compromised in MMR. This impairment, however, appears selective because sensitivity to other visual attributes, such as contrast, falls within normal limits. To explore the degree to which the impairment can be related to visual rather than response difficulties and to investigate sensitivities to other fundamental visual attributes, we designed the ability of MMR and nonretarded adults to detect forms defined by texture and motion. Using signal detection methodology, subjects viewed a randomly textured and moving background and were instructed to indicate the presence or absence of these forms. Texture differences were introduced by varying the grain size of the form relative to the background. Motion differences were introduced by varying the correlation with which the texture elements were displaced across temporal frames. Analyses of d' and response bias indicator that the MMR encountered difficulty in veridically perceiving motion information, (2) both groups responded similarly to texture information, and (3) the impairment in perceiving motion noted in MMR cannot be simply ascribed to response difficulties. These results are discussed with respect to the possible genetic, extent, and impact of these selective impairments.

Supported by HD15051, HD27716, and EY00590

586.4 MOVEMENT IN ORTHOGONAL DIRECTIONS: DISCRIMINATION BY MENTALLY RETARDED AND NONRETRARDED ADULTS. *Sandy A. Shimp, Robert Fox, & Stephen Gross.* Department of Psychology, Vanderbilt University, Nashville, Tennessee 37240.

Our prior research has demonstrated that the perception of motion-defined forms is impaired in mildly mentally retarded adults (MMR). The existence of perceptual deficits should have implications for the possible nature and degree of visual impairment. Interestingly, multiple sclerosis patients are impaired at perceiving motion-defined forms yet are able to veridically discriminate between different directions in a single visual field. One hypothesis is that the perception of motion-defined forms and direction of motion involve different neural structures. The present study investigated the ability of MMR and nonretarded adults to discriminate between movement in orthogonal directions. Subjects were asked to discriminate between random-element kinematograms moving either up/down or left/right. The coherence of the motion in the kinematograms was manipulated by varying the correlation (ranging from 0 to 100%) with which the elements were displaced across temporal frames. Both MMR and nonretarded adults were essentially equivalent in their ability to discriminate between the directions. The results are similar to those found with multiple sclerosis patients and are consistent with the explanation relying upon different neural structures. Alternative hypotheses, however, are available that can account for these results. One considers the signal/noise ratios in displays used to test the perception of motion-defined forms and directions of motion. Another postulates that differences between groups would be detected if smaller differences in direction were used. We are currently exploring the validity of these alternatives.

Supported by HD15051, HD27716, and EY00590

586.5 LACK OF VEP EVIDENCE FOR MAGNOCOCCULAR DYSFUNCTION IN DYSLXIA. *J. Victor, M. Conte, L. Burton, and R. N. Nass.* Dept. of Neurology and Neurosciences, The New York Hospital - Cornell Medical Center, New York, NY (1002).

It was recently reported (Livingstone et al., PNAS, 1991) that five dyslexic individuals showed less of the pattern-reversal visual evoked potential (VEP) under conditions designed to isolate the magnocellular pathway: high temporal frequency, low contrast, and low luminance. We measured contrast-reversal VEPs in dyslexic individuals aged 8 to 40 and in 11 controls with learning disability but not dyslexia, 7 normals of similar age. For the purpose of this study, dyslexics were defined as subjects of normal intelligence with a history of reading difficulty. We used steady-state conditions similar to those used by Livingstone et al. (16 Hz reversal rate, 2% contrast, 4 cd/m², 3 deg checks), and an analog method (Victor and Mast, ERG J., 1991) which provided rigorous statistical criteria for detection of significant responses and significant differences between responses. Given the findings of Livingstone et al., we found that VEPs were absent in most subjects not only in the dyslexic group, but also in the control group. At lower temporal frequencies (4 and 8 Hz reversal rate), higher luminance (59 cd/m²) and higher contrast (20%), significant responses were more widely present in both groups, but the response amplitudes and phases did not differ across groups. We find no evidence for a low-contrast, low-luminance VEP abnormality associated with dyslexia.

Supported by EY7977 (N).

586.6 PUPILLARY LIGHT REFLEX (PLR) AFTER OPEN LOOP RETINAL STIMULATION IN HUMANS. *W.B. Pickworth*, J.S. Fossnaugh, J.D. Nichols and E.B. Bunker. NIDA, Addiction Research Center, Baltimore, MD 21224.

Pupil diameter (PD) and the PLR are popular, noninvasive measures of drug action. Ambient light level and an opaque patch over the contralateral eye significantly change prestimulus PD and PLR in closed loop experiments (Pickworth et al., Soc Neurosci Abs 17:1467, 1991). In the present study, ambient light and the stimuli were presented in Maxwellian view (open loop) to nine drug-free volunteers using a Pulse Medical Instruments photometer. The PLR was evaluated at four levels of ambient light: with both eyes open, a patch over one eye. In both patch conditions, increased ambient light decreased PD from 6.4 to 4.2 mm and constriction velocity (CV) from 6.3 to 3.4 mm/sec. These results differ from the closed loop experiment where the patch doubled CV and increased PD by 50%. Under Maxwellian view binocular summation is eliminated suggesting that midbrain control of PD is diminished.

586.7 AUGMENTING AND REDUCING OF THE VISUAL EVOKED POTENTIAL IN RONAL HIGH- AND LOW-AVOIDANCE RATS. *J. Figiel and P. Eisenberg.* School of Life and Health Sciences, Unv. of Delaware, Newark, DE 19716 and P. Briggson, Laboratory of Behavioral Neurology, ETH, Zurich, Switzerland.

In humans and cat high avoidance, theta (as SSD) tend to show decreasing amplitudes (augmenting) of the P1 and N1 components of the visual evoked potential (VEP) to increasing intensities of light flash, whereas low SSDs show VEP rising (Verh.) and Roman low-avoidance (RLA/Verh.) bred in Switzerland, have behaviorally been comparable to high, high, and low SSDs, respectively. RLA/Verh. rats show greater exploration, activity, and aggression than do RHA/verh. rats. Male rats of each strain were anesthetized with chloral hydrate and maintained at a stable moderate anesthetic level. Fifty VEPs per vertex at each of five flash intensities were computer averaged per rat. The slopes of P1 and P3 (mV) amplitudes as a function of flash intensity were significantly greater in the RHA/verh than the RHA/verh was higher than the RLA/Verh and had almost flat amplitude-intensity functions.

This study demonstrates a rat model of SS-related augmenting and reducing that yields advantages of genetic homogeneity and a short gestational time, and provides access to a wealth of behavioral data and experimental manipulations available for the cat. We have shown that this relationship has a heritable base and extends across species from human, cat, and rat. (Supported in part by ARO Contract DAAL 038880043)


We performed a study to investigate the feasibility of a visual prosthesis for the blind based on electrical stimulation of monocular microelectrodes inserted in a blind volunteer. A healthy, 42 year old woman with a 22 year history of bilateral complete blindness secondary to glaucoma was selected based on a rating system involving eye history, examination, and willingness for surgery. The experimental and time-limited nature of this first study was explained in detail to the patient volunteer who gave her consent.

The surgical procedure was designed based primarily on the fragility of the electrodes and the complex anatomy of the occipital pole region. A strain relief system utilizing silicon tubes and flanges was devised to minimize movement of the electrodes. Parylene insulated iridium "hat-pin" microelectrodes (n=30) with attached gold leads were manually implanted 2mm deep in the visual cortex near the occipital pole. The leads exited the craniotomy site via separate contoured rumps in the skull and penetrated the scalp through four separate incisions. The occipital cortex was stimulated in daily sessions over four months with occasional respite. Stimulation was never associated with pain or any other discomfort. The sensations produced were typically described as small spots of light varying in color, depth, position, and intensity. Percept qualities could be modulated by adjustment of stimulation parameters, including current amplitude, frequency, pulse duration and train length. Breakages occurred in some electrode leads, which we feel can be prevented with future designs.

The leads and several microelectrodes were removed when testing was completed. There has been no significant morbidity associated with this study.
586.9

Using intracortical microstimulating electrodes, cortical phosphenes were produced at the lowest stimulus levels ever reported in humans (see preceding abstracts for details). Reliable and rapid recognition of visual images made up of groups of electrically elicited phosphenes is the most important goal yet to be achieved in developing a visual prosthesis for the blind. Breakage of leads in a patient-volunteer who had been blind 22 years limited the simultaneous activation of multiple intracortical microelectrodes and resulting phosphenes to six in a vertical row which could be recognized as a letter ‘T’.

To further evaluate pattern recognition, a visual cortical implant system with over 200 intracortical microelectrodes arranged in pairs is being developed. In the next, more recently blinded patient-volunteer a percutaneous connector rigidly mounted to the skull is planned. Also a television camera and image processing electronics will be used to provide visual images in addition to computer generated patterns. In a definitive prosthesis a multichannel transcutaneous telemetry system will be needed.

586.11

Sequential and simultaneous stimulation of microelectrodes implanted in the area of the visual cortex of a blind patient-volunteer (see preceding abstracts for details) produced multiple phosphenes in the perceived visual field. Phosphene locations were mapped using subject descriptions, a dart board mapping procedure or a pair-wise computer mapping algorithm. All phosphenes were located in the left hemifield with most near or above the horizontal meridian within 40 degrees of the fixation point. The apparent distance at which phosphenes appeared ranged from a few inches to the location of a “distant star.” When multiple phosphenes were produced simultaneously they tended to look alike and appeared at the same depth. When the stimulating current was increased above threshold on some electrodes, a second phosphene appeared in close proximity to the first phosphene. Multiple phosphenes, produced with either single or multiple electrodes, maintained their relative positions as they moved with conjugate eye movements. The relative relationship of the phosphenes in visual space was roughly similar to the placement of the electrodes in the visual cortex.

The apparent spatial concentration of the phosphene map may be due to the anatomical placement of the intracortical electrodes, the length of blindness (22 years) or the type of blindness (glaucoma).

587.1
A DYNAMICAL MODEL FOR COMPUTING THE POSITION OF AN OBJECT FROM ITS RETINAL LOCATION AND EYE POSITION. A. Pouget, T. Allibert* and T.J. Serowowski. The Salk Institute, La Jolla, CA 92037.

The position of a visual object with respect to the viewer, the egocentric position, can be computed by adding the retinal position of the object with the position of the eyes. As experiment performed by Matin et al. (Science, 248, 1960) suggests that humans use this simple algorithm when localizing visual objects. The task was to localize a point of light briefly flashed (<1ms) on a screen while a subject’s eyes were fixated at a point. The subject was asked to report the position of the light in the absence of any other visual stimuli which suggests that the eye position was apparently available to the observer. Additional experiments, however, showed that humans tend to make a systematic localization error in the direction of the eccentricity (Masef, Psych. Perc., 24, 1978). Furthermore, the size of the error was a function of the retinal position on which the flash implied (O’Ragan, Psych. Perc., 36, 1984). Thus, the perceived egocentric position of an object is not a static linear sum of the retinal position and eye position.

We have developed a biologically plausible model that computes egocentric position by dynamically combining the retinal position of an object with eye position. The model accurately locates an object when it is presented for a long duration (>100 ms) with the eyes at rest. However, for brief presentations while the eyes are moving, the model exhibits a pattern of errors identical to that reported in humans. Taken together with recent physiological and psychophysical results (Gauthier et al., Science, 249, 1990), our model suggests that the eye position is used for object localization. (Supported by the Howard Hughes Medical Institute).

587.2
THE QUANTUM EFFICIENCY OF HUMAN VISION: COMPARING PSYCHOPHYSICS & ANATOMY. Denis G. Pelli* Institute for Sensory Research, Syracuse University, Syracuse, NY 13244-5290.

Transduction efficiency (fraction of conical photons that produce an isomerization) has received much attention since the time of Hecht, but is still subject to much uncertainty. Pelli (1990) introduced a new psychophysical technique that measures transduction efficiency by measuring the effects of visual noise masking. The X’s show preliminary measurements indicating peak transduction efficiencies of 5-10% for both rods and cones. They agree with anatomical estimates shown as curves, which are computed from the known number of receptors and their optical densities and aperture sizes.

THURSDAY PM
VISUAL BEHAVIOR: PSYCHOPHYSICS AND EYE MOVEMENTS

S87.3
A NETWORK MODEL OF DEPTH CUE INTEGRATION. M.B. Lader* and D. Zipser. Department of Cognitive Science, UC San Diego, La Jolla, CA 92039-0515.

Many different cues give information about depth, but it is not known how they are integrated to form unified perceptions. To investigate this, we trained neural network models with biologically realistic visual input to produce depth maps of the same objects used in psychophysical experiments on the integration of binocular depth cues (Bulthoff and Mollon, J. Optical Society of America, A5:1749-1758, 1988). In the psychophysical study, subjects judged the depth of points on computer generated objects with various combinations of shading and stereo cues. Their study showed that perceived depth decreases with fewer cues. Our network models were trained to estimate the depth at the center of its binocular receptive field. The use of targets corresponding to psychophysically measurable quantities for network training ensures that network responses can directly compare human responses. Networks trained solely on cue-rich images show a systematic decrease in "perceived depth" to cue-impoveryished images similar to that found in humans. Differences between human and network responses highlight global-local conflicts in depth perception. By analyzing how these model networks integrate different depth cues, hypotheses can be generated as to how this integration takes place in the brain. These results extend the neural systems identification paradigm (Zipser, Neuroscience, 47(4):853-862, 1992) to models of psychophysical data.

This research was funded by NIH Grant MH45271 and System Development Foundation Grant G339D to DZ.

S87.5
VISUAL AFTEREFFECTS IN THE MONKEY: THE JUDGEMENT OF VERTICAL AFTER ADAPTATION TO OBLIQUE CONTOURS.

In humans, it has been shown that the subjective estimate of the orientation of a contour is shifted by several degrees from its real orientation after extended viewing of slanting contours (even if a shift of several degrees from the orientation of the contour to be judged. This phenomenon, called the tilt aftereffect (TAE), has frequently been used in studies attempting to determine the neural substratum of contour perception. As a preliminary to making direct studies of neurons that might participate in the TAE, we trained a monkey with an implanted eye coil to fixate a central target (95% contrast square wave grating) and report the orientation of a subsequent, briefly presented (100ms) test target (20% contrast square wave grating of various orientations) by making a leftward saccade to indicate a counterclockwise (CCW) orientation and a rightward saccade to indicate a clockwise (CW) orientation. After learning the task, the fixation time at the start of each trial was gradually increased to 5 secs, and a 600 ms blank interval introduced before each test target presentation. The proportion of test orientations judged to be CW were used to construct psychometric functions and showed that the monkey's estimate of subjective vertical (orientation at which CW or CCW choices were 50%) was dependent upon the orientation of the grating viewed during the fixation interval. Fixation grating tilted 15 deg. from vertical produced a shift of 1-1.5 degs in subjective vertical, while fixation gratings oriented vertically or horizontally produced no shift. The TAE observed was slightly smaller than that shown by human subjects tested under the same conditions. The presence of a TAE in monkeys rules out many cognitive explanations for the effect, and demonstrates the feasibility of quantitative investigations of perceptual effects in animals. 1Supported by leave from Florida State Univ., Tallahassee, FL.

S87.6
A NEW EFFECT IN COLOR AND SPATIAL VISION: VISIBILITY OF A TEST PATTERN IN A MASK IS BANDPASSED WITH VIEWING DISTANCE.

We observed that a masked pattern was often most visible at intermediate viewing distances. We examined this masking effect as a function of the mask's relative spatial frequency (SF) and relative orientation in both chromatic and achromatic modes. A spatially localized test target (e.g. 1 cpd at 80 cm with 30% contrast) and sinusoidal mask were displayed on a Sony GDM1936 color monitor interfaced with AT'Vista Graphic System was used. The Red-Green (RG) channel was isolated by the minimum flicker equiluminant criterion and the hue cancellation technique. Two non-obscureable magnitude estimations (ME) using ratings between 0 (test pattern was not seen) and 10 (test was most distinctly seen) were found bandpass ME visibility curves (ME rating vs. viewing distance) for both achromatic (reddish and greenish patterns inphase) and RG chromatic (patterns antiphase) modes for vertical test and masks oriented 14.5°, 45° and 90° from the vertical. The visibility of the test pattern increased as the orientation of the mask relative to the test increased at a given distance. Our data showed that the visual system has SF and orientation tuned mechanisms at suprathreshold contrasts (similar to threshold contrasts) in both chromatic and achromatic modes. Low test pattern visibility suggested that the test was processed mostly by the same SF and orientation tuned mechanisms as the mask whereas higher pattern visibility indicated processing by different mechanisms. This masking effect, which is stronger in the chromatic mode, can be explained by SF and orientation tuned mechanisms, and by the apparent contrast of the mask relative to the test at different viewing distances.


S87.7
SPATIAL SUMMATION OF VISUAL INFLUENCE ON EGOCENTRIC LOCALIZATION: VARIATIONS WITH BINOCULARITY AND REPORTING MODE.

The elevation of a target set to appear at visually perceived eye level (VPEL) varies linearly with the pitch of a vertical line of variable length was measured at 2 eccentricities (ecc), 5° and 25°; slopes of VPEL/Pitch functions increased exponentially with eccentricity with slopes at eccentricities of 5° and 15°, respectively. For the shortest line (3°) pitch was 33% more potent at 5° ecc, but this predominance decreased and reversed with longer lines so that at 65° ecc the more peripheral stimulus was 55% more potent. Max. VPEL/Pitch slopes were 0.36 and 0.55 at 5° and 25° ecc, respectively. It is not evident that a shift relative to retinal orientation of the parafoveal line than the peripheral line.

The relative effectiveness for egocentric localization of short and long lines in the parafovea as compared to the periphery and the variation in space constant is in a similar relation to that found for visual acuity and for V1 receptive field size. However, the 15° space constants for VPEL are at least an order of magnitude larger than appropriate for visual acuity or for V1 receptive field size, but they do correspond to receptive field sizes reported in MT, MST, and Ta.

(Supported by AFSOS 91-0146)
CHARACTERIZATION OF THE POTASSIUM CURRENTS IN TYPE I MAMMALIAN AND AVIAN SEMICIRCULAR CANAL HAIR CELLS. Reiss et al. The University of Texas Southwestern Medical Center, Dallas, TX 75235 marks.

Type I vestibular hair cells differ in their ionic currents from type II hair cells. Isolated pigeon type I cells are known to have significantly lower input resistance than type II cells and a potassium current active above -80mV. We have used the perforated patch technique to make whole-cell recordings from type I hair cells, in order to further identify the currents. Cells were dissociated from the semicircular canals of pigeons. Two potassium-selective conductances and a putative non-selective cation current modulated by acetylcholine (100 μM) have been found so far. At potentials above -40mV, one of the potassium currents is first present. This current is sensitive to externally applied 100μM quindine, 4mM barium and 37μM nifedipine, properties consistent with k being a calcium-activated potassium current. At potentials positive to -80mV the other potassium current is present and is blocked by externally applied 40μM nifedipine. Large inward tail currents (approx. 80pA) associated with this conductance appear to lead to cell membrane depolarization. This current is sensitive to calcium, being reduced by externally applied nifedipine (100μM). Depletion and removal of external calcium. At the average zero-current potential in gel hair cells (-61mV), this current is 90% activated. Supported by NIH grant DC00273.

CROSSLINKING OF VANADATE-TRAPPED NUCLEOTIDES TO TWO MYOSIN CANDIDATES IN HAIR BUNDLES. P. G. Gillespie* and A. J. Hindsg leth. Department of Biophysics and Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75235-9039.

Our model for mechano-electrical transduction by hair cells (Hudspeth, A. J. 1989 Nature 342: 597-604) invokes an active process, perhaps a member of the myosin family, to account for adaptation of the receptor current. Because direct evidence for a hair-bundle myosin is lacking, we sought candidate molecules by asking which constituents of purified hair bundles bind nucleotides under circumstances characteristic of myosins. Irradiation with ultraviolet light efficiently crosslinks vanadate-sensitive nucleotides to many myosins (Murata and Korn, E. D. 1981 J. Biol Chem. 256: 499-502). After exposure to [α-32P]ATP or [α-32P]UTP, photoscanning labeled proteins of 120 and 230 KD in hair bundles isolated from the bullfrog’s sacculus. Vanadate-trapping experiments were consistent with their being myosins. After forming a complex with myosin and newly hydrolyzed ADP or ATP, vanadate dramatically decreases the dissociation rate of the bound nucleotide. We found that vanadate required for high binding of nucleotide to the 120 and 230 KD proteins. We could eliminate labeling of the two bundle proteins by simultaneous incubation with an excess of unlabelled ATP (not ADP) for 45 minutes (reaction time for ATP was 10 minutes). A divalent cation (Mg2+ or Mn2+ but not Ca2+) was required for observation of labeled proteins. The myosin candidates were surprisingly resistant to solubilization by n-octylglycoside (a detergent that removes actin). In contrast, with this observation, we detected no biotinylated proteins in ATP extracts of isolated hair bundles. We surmise that the 120 and 230 KD proteins are myosins that are tightly attached to a cytoskeletal structure. This research was supported by NIH grant DC00241.

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Macroscopic currents were recorded from dissociated rat utricular hair cells using the whole-cell tight-seal patch-clamp technique. The average zero-current potential was -14 ± 3.1 mV. Hyperpolarizing steps from the holding potential of -61 mV elicited an inwardly rectifying current (I_{IR}) that was reversibly blocked by 5 mM external Cs, suggesting a K^+-selective current. The slope conductance was 34 ± 2.7 nS/mV, but the current was variable (7.4±1.1, mean±SD). Block of I_{IR} by Cs showed that I_{IR} is K-selective. The lack of N-shape in most current-voltage (I-V) curves suggests that Ca-dependent current is not present. The rank order of potency for reducing 2 outward K currents, delayed rectifier and a Ca current, is 100 mM 4-AP > 1 mM AP5 > 100 μM CNQX > kynurenic acid. The rank order of potency for reducing 2 inward K currents, delayed rectifier and a Ca current, is 100 mM 4-AP > 1 mM AP5 > 100 μM CNQX > kynurenic acid. CNQX and kynurenic acid exerted a non-inactivating inward current. With internal Cs to block K currents, depolarizing steps positive to -40 mV elicited inward currents that peaked at 0 mV. These are likely to be Ca currents.

ACETYLCHOLINE MEDIATED CURRENTS IN SOLITARY TURTLE COCHLEAR HAIR CELLS. M. B. Goodman* and J.J. Art, Committee on Neurobiology and Dept. of Pharmacology & Physiology. The University of Chicago, Chicago, IL. 60637.

The efferent post-synaptic potential in turtle hair cells consists of a fast depolarization followed by a longer-lived hyperpolarization. Acetylcholine (ACh) mediates a transmitter at the efferent hair-cell synapse. In the turtle, the entire post-synaptic potential is blocked by atropine and d-tubocurarine. In addition, superfusion with ACh hyperpolarizes the hair cell and eliminates its response to a subsequent efferent stimulation (Art, Fettplace & Fuchs, 1984, J. Physiol. 365, 526-550). The underlying ionic conductances have been studied in solitary hair cells exposed to ACh. Rapid superfusion of ACh produces a net outward current (20-100 pA) in most cells voltage-clamped at their resting potential (22 of 45). The I-V relation of these cells is most simply interpreted as an increase in the amplitude of I_{K(Ca)} active at all potentials depolarized to rest. This current may reflect a global increase in intracellular Ca^2+, which declines in the continued presence of 50% of total generated a fast inward current followed by an outward current when exposed to ACh and voltage-clamped at rest. The time course of the response in these cells is similar to the potential observed in the turtle half-head. The I-V curve for the late component reverses near the potassium equilibrium potential, consistent with the notion that an additional K current mediated by the ACh receptor

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SYNAPTIC PLASTICITY IN VESTIBULAR HAIR CELLS OF ANIMALS EXPOSED TO MICROGRAVITY. M.D. Ross and T.C. Chimeno
Bioimaging Center, NASA, Ames, CA 9329-11 Moffett Field, CA 94035-1000
Do synapses in hair cells change in number, size or type as a result of exposure to altered gravity during space flights of short duration? This report focuses on results obtained from three maculas of rats flown for nine days aboard the Spacelab Life Sciences 1 shuttle, and from three matched ground controls. Tissues were obtained within six hours of landing. Fixed, post-osmicated, dehydrated, microdissected, and prepared for transmission electron microscopy according to usual methods. Serial sections (0.2 μm) were cut from the medial border of the macula. More than 600 hair cells, containing more than 300 ribbon synapses, were studied. Synapses in type I hair cells of flight animals increased by 41% compared to type I hair cells of ground controls, and synapses in type II hair cells increased by 57%. This increase was greater in spherules than in rod synapses. There was a significant rise in spherules in both type I (5%; p < 0.05, N=301) and type II hair cells (74%; p < 0.005, N=330). Most of the additional spherule synapses were present in multiples. In type I hair cells the number of synapse pairs increased from 9% in controls to 18% in flight animals, and in type II hair cells the number of synapse pairs increased from 19% to 52%. Groups of 3-6 spherule synapses in type II cells increased from 2% to 21%. The more numerous multiple synapses in type II cells contacted collaterals rather than ciliae. The size of synapses was not significantly different between flight animals and ground controls. The results indicate that macular hair cells exhibit plasticity and that the macular neural network can adapt to an altered gravitational environment.

This work supported by National Aeronautics and Space Administration.

IMMUNOHISTOCHEMICAL LOCALIZATION OF S-100 PROTEINS IN AUDITORY AND VESTIBULAR END ORGANS OF THE MOUSE. I.G. Foster,2 M.J. Drucker3 and T.C. Chimeno2,3,4
1. Lab of Bio-technology, Dept. of Biological Sciences, Wayne State University, Detroit, MI 48201.
Calcium-binding proteins are thought to be involved in the transduction of intracellular calcium signals which mediate cellular responses to extracellular events. In auditory and vestibular end organs, calcium-binding proteins may modulate certain calcium-dependent processes. Utilizing immunohistochemical procedures, we have localized the mouse in the mouse labyrith 1-500-like immunoreactivity, the S-100 proteins representing one subfamily of the EF-hand family of calcium-modulated proteins.
Cochlear inner hair cells and neural elements of the spiral ganglion were weakly immunoreactive. The cytoplasm of Deiters' cells and the supranuclear regions of outer hair cells were strongly positive for S-100-like immunoreactivity, while the supranuclear regions of outer hair cells were unreactive. Fibers in the spiral ligament were strongly immunoreactive.
In the vestibular end organs, including the saccule, utricle, and semicircular canals, nerve fibers underlying the sensory epithelium and nerve calyces surrounding type I hair cells were immunoreactive. Types I and II vestibular hair cells appeared weakly immunoreactive, while epithelial supporting cells showed no immunoreactivity.
The localization of S-100-like immunoreactivity to the basal regions of cochlear outer hair cells and to presumed afferent nerve axons underlying vestibular type I hair cells is consistent with a functional role for S-100 proteins at these sites in the regulation of calcium-dependent events.
(Supported by NIH Grants DC 00356 and DC 0056.)

REGENERATIVE CELL PROLIFERATION IN THE MAMMALIAN UTRICLE. A. Lysakowski, Dept. of Pharmacology, School of Medicine, University of Chicago, Chicago, IL 60637.
Postembryonic proliferation of supporting cells, which leads to the addition of new hair cells, has been demonstrated in the inner ear sensory epithelia of fish, amphibians, and birds. The vestibular sensory epithelium in mammals share most morphological traits with their counterparts in those groups, but cell proliferation in mammalian vestibular epithelia has not been thought to occur only during embryonic development. We have tested this assumption by explanting utricles into culture and have observed that regenerative proliferation of mammalian supporting cells can occur.
Utricles were removed from juvenile and sexually mature albino guinea pigs (wt: 400-700 gm), and cultured in Rose chambers at 37°C. Hair cells were lesioned by incubating the cultures in media that contained ototoxic antibiotics (either 0.5-1.0 mM neomycin or 5-10 μg/ml of sodium chlorate). These mitochonria (0.24 ± 0.06 μm diameter) and thicker stereocilia. This second class was found in units identified as dimorphic units by the presence of calyceal collaterals. Type II hair cells had subcuticular mitochondria similar in size to the second class. We have confirmed this difference in several IRP-labelled calyceal and dimorphic units. Further confirmation will be obtained using an antibody to calretinin, a calcium binding protein apparently found only in calyx units. (Supported by RO3-DC01474.)

POSSIBLE MORPHOLOGICAL FEATURES TO DISTINGUISH CALYX VS DIMORPHIC TYPE VESTIBULAR HAIR CELLS IN THE CHINCHILLA CRISTAE. A. Lysakowski, Dept. of Pharmacology, and Physiol. Sciences, The University of Chicago, Chicago, IL 60637.
A type I vestibular hair cell is enclosed within an end Mafia. A calyx, however, can belong to either a dimorphic unit or to a pure calyx unit (Fernández et al., J. Neurophysiol. 60:167-181, 1988). Previously, it has been difficult to distinguish type I hair cells belonging to pure calyx or dimorphic units from those belonging to dimorphic units without some form of dye labeling of the afferent fiber. Recently, however, we have observed some morphological features which may serve to distinguish the two types in unlabelled material. Based upon serial section analysis of ten samples from cristae in four animals, there appeared to be at least two classes of type I hair cells. The first class has large mitochondria (0.52 μm ± 0.23 in diameter) subjacent to the cuticular plate and thicker stereocilia. These mitochondria were twice as large as those found elsewhere in the same cell. Cells containing these larger mitochondria were found mainly in the central zone at the apex of the crista and occasionally in the intermediate zone. So far they have not been observed in the peripheral zone. The second class of type I hair cells has smaller subcuticular mitochondria (0.24 ± 0.06 μm diameter) and thinner stereocilia. This second class was found in units identified as dimorphic units by the presence of calyceal collaterals. Type II hair cells had subcuticular mitochondria similar in size to the second class. We have confirmed this difference in several IRP-labelled calyceal and dimorphic units. Further confirmation will be obtained using an antibody to calretinin, a calcium binding protein apparently found only in calyx units. (Supported by RO3-DC01474.)

RECEPTION OF LOW INTENSITY MILLI-METER-WAVE RADIATION BY SENSORY ORGANS. G. N. Akory, V. D. Avet'kov, P. G. Semenikov and R. N. Slob*, I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia, Kara-Dag Biological Station, Crimea, Ukraine, and Department of Physiology & Biophysics, University of Tennessee, Memphis.
The effect of low intensity millimeter-wave electromagnetic radiation at a power intensity of 1.5 mW cm2 to the duct opening at 1-10 mm distance caused transient increases in the firing rates of single afferent units, followed by adaptation during 2-5 min to the initial level. The reverse effect was observed after offset of irradiation. When the power intensity was increased, inhibitory responses were also observed. The inhibitory responses were more pronounced in receptors with higher electrical thresholds; these did not show any excitatory response. On the contrary, on irradiation from a distance of 15-20 mm from the duct opening, the receptors responded with a prolonged excitatory activity lasting up to 20 min. Direct irradiation of the sensory cells irrespective of their electrical threshold produced only an inhibition. The maximal effect of millimeter-wave radiation was shown to be at 55 GHz. The mechanism of the effect of low intensity millimeter-wave radiation on the amplitudes of Lorenzini will be discussed.

THE ORGAN OF CORTI RESPONDS TO RETINOIC ACID (RA) AND CONTAINS RA, A NUCLEAR RA-RECEPTOR, AND CRABP DURING THE PERIOD OF HAIR CELL DEVELOPMENT. M.W. Kelley, X. M. Su, M.A. Wagner* and J. Conley*. Dept. of Otolaryngology-HNS and Dept. of Neuroscience, University of Virginia, Charlottesville, VA 22908.
Postembryonic proliferation of supporting cells, which leads to the addition of new hair cells, has been demonstrated in the inner ear sensory epithelia of fish, amphibians, and birds. The vestibular sensory epithelium in mammals share most morphological traits with their counterparts in those groups, but cell proliferation in mammalian vestibular epithelia has not been thought to occur only during embryonic development. We have tested this assumption by explanting utricles into culture and have observed that regenerative proliferation of mammalian supporting cells can occur.
Utricles were removed from juvenile and sexually mature albino guinea pigs (wt: 400-700 gm), and cultured in Rose chambers at 37°C. Hair cells were lesioned by incubating the cultures in media that contained ototoxic antibiotics (either 0.5-1.0 mM neomycin or 1.0 mM gentamicin). Then the cultures were incubated for 2-6 days in aminoglycoside-free media that contained either β-methyl-thymidine (0.8 μCi/ml) or bromo-deoxyuridine, so that DNA would be labeled in any cells that had proliferated during the culture period.
At least four labeled cell nuclei were present in the sensory epithelium in each case. The occurrence of the labeled cells demonstrates that mammalian hair cell epithelia can respond to trauma by renewed proliferation. In addition, these results and others (Forge et al., submitted) suggest that mammalian vestibular organs are capable of a heretofore unexpected degree of self-repair after hair cell loss.
(Supported by funds from the NICDD and the LOVHF.)

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EXPRESSION OF A PROTEIN WITH EGF-LIKE IMMUNOREACTIVITY IS TRIGGERED BY TREATMENTS THAT EVOKE HAIR CELL REGENERATION. K-M. Xu, and J.T. Corwin.* Dept. of Otolaryngology-HNS and Dept. of Neuroscience, Univ. of Virginia, Charlottesville, VA 22908.

We have used the trauma-evoked response of the avian cochlea to investigate biochemical changes that lead to regeneration of hair cells. Multiple experimental groups of 7 to 9-day-old white leghorn chicks were each given an injection of gentamicin (100 µg/kg body weight) and then exposed to tone burst sound stimulation at 1.5 kHz and 120 dB SPL for 24 hr, so as to damage hair cells and trigger regeneration in the cochlea. Immediately after the treatment, the chicks were euthanized and their cochleas were removed. The sensory epithelium was dissected from each and homogenized in a detergent buffer containing protease inhibitors. Supernatants were run through SDS-PAGE under reducing conditions along with samples from age-matched controls. Nitrocellulose blots were reacted with a polyclonal antibody raised against epidermal growth factor (EGF). Our preliminary findings indicate significant anti-EGF labeling of a protein that runs at approximately 14 kD in all samples from the experimental groups. The strong labeling of this protein in samples from treated cochleas contrasted with an absence of labeling or barely detectable labeling at that position in control lanes. Bands at higher molecular weights were comparably labeled in both controls and experimental, providing an internal control for the assay. The increased expression of this protein is detectable immediately after a treatment which causes extensive hair cell loss and which evokes hair cell regeneration. This suggests that an EGF-like domain may participate in the cascade of events that initiates the replacement of hair cells. (Supported by grants from the NIDCD and the NOFHR)

RAT UTRICULAR MACULA: BLOOD FLOW AND STEREOLOGICAL ASSESSMENT OF CAPILLARY MORPHOLOGY. M.J. Lyon* and R. Payman.

Dept. of Otolaryngology, SUNY Health Science Center, Syracuse, NY 13210

Vascular compartments have long been proposed as a cause for inner ear disorders. However, the examination of blood flow and its control mechanisms in the vestibular system has been very limited. Combining stereological techniques with the microscope injection technique, capillary morphology and regional blood flow were determined for the young adult rat utricular macula. Results are: total utricular blood flow, 0.1581 ±0.0783 μl/min; blood flow to the neuroepithelium (excluding nerve), 0.0956 ±0.0466 μl/min; blood flow/unit volume, 7.71 ±4.31 μl/mm3; neuroepithelial volume, 0.0132 ±0.0018 mm3; capillary surface area, 11.75 ±1.78 mm2/unit volume; mean capillary diameter, 5.84 ±0.56 μm; capillary length/unit volume 627.4 ±78.0 mm/μm3; volume fraction of capillary lumens, 0.018 ±0.004. Comparisons to data for the posterior canal ampulla (Wanamaker and Lyon, Otolaryngol Head Neck Surg 103:556, 1990) indicate that the mean capillary diameter in the rat utricular macula is smaller; the capillary length/unit volume is greater; the end organs are similar with respect to neuroepithelial volume, capillary surface area/unit volume and blood flow/unit volume. The size of the microsphere used in the present study (9.21 μm), in comparison to the mean capillary diameter (5.84 μm) of the utricular neuroepithelium would indicate that the blood flow data likely represents a minimum value. These findings indirectly indicate that the metabolic rate of the utricular macula is greater than that of the posterior canal ampulla. If there is as much variation in capillary diameter between the neuroepithelium of the other end organs then regional vascular blood flow studies would have to be performed using microspheres sized appropriately for each end organ.

EFFECTS OF SOURCE DISTANCE ON THRESHOLD DETECTION AND SOURCE LEVEL DISCRIMINATION BY THE LATERAL LINE OF THE MOTTLED SCULPIN, G. Columba. Paralyzed Hearing Institute, Loyola University of Chicago, IL 60626.

The feeding response of the mottled sculpin was used to measure (1) threshold levels of detection, and (2) 50 Hz vibration (diameter) spherical source and (2) just-detectable level increments in on-going 50 Hz vibrations of the same source placed near the head or trunk of the fish at varying distances. At distances between 15 and 60 mm, source level at mean threshold detection (4 fish) increased with distance at an average rate of around 14 dB/distance doubling. Mean level discrimination limens (5 fish) for equivalent 10 dB sensation levels did not change with distance and were around 6.5 dB. These results show that (1) incomparable flow fields (falling off at 18 dB/distance doubling) are more likely to govern detection responses than pressure (falling off at 6 dB/distance doubling), but that (2) spatial patterns of flow amplitude, which change dramatically with distance near the source, do not affect the ability of mottled sculpin to discriminate source levels.

HAIR CELL AND SYNAPTIC RECONSTRUCTIONS IN THE AMPHIBIAN PAPILLA IN LEOPARD PIPPIES, PIPPIES, P. C. Bertolato, D.D. Simmons, M. Leong, and P.M. Narins.* Dept. of Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024

Amphibians have two auditory organs in each inner ear that are specialized for the reception of airborne sound: the amphibian papilla (AP) and the basilar papilla. In the leopard frog, the AP is innervated by fibers with characteristic frequency (CF) ranges from 100 – 1250 Hz. The AP is believed to be tonotopically organized. To address ultrastructural differences in different parts of the AP, hair cells and their synapses were reconstructed from plastic cross sections. Hair cells from the rostral AP had a mean length of 43.1 ± 11.3 μm and a mean area of 292 ± 67 μm². Although the lengths in both caudal and rostral extensions vary significantly, within each region the length decreases along the posterior to anterior axis. In the rostral AP hair cells had 9 to 11 afferent synapses and at least one efferent synapse. In contrast caudal AP hair cells had only 3 to 5 afferent synapses and efferent synapses were not readily observable. Although the present light shows that there are definite morphological correlates of a tonotopic organization, these data additionally suggest that there may be fundamental differences between the rostral and caudal portions of the AP. (This research was supported by NSF grant BNS9110694 to DDS and NIH grant DC00222 to PMK.)


The sensory structures of the inner ear are difficult to preserve with conventional fixation methods because such structures are surrounded by bodies of fluid (endolymph and perilymph). Even intracardial perfusion can lead to diffusion of molecules from cells and tissues, thus producing poor fixation. This study looks at microwave irradiation as a possible alternative method for fixation of sensitive tissues. The structural preservation of vestibulocochlear sense organs is compared to conventional fixation methods which either a) immersion in primary fixative combined with microwave irradiation; b) intracardiac perfusion combined with microwave irradiation; or c) conventional fixation methods of immersion without microwave; and d) conventional perfusion without microwave irradiation. Sixteen temporal bones (TB) were harvested from eight chicks and immersed in 10% buffered formalin, followed by 10% buffered formalin and microwave irradiation. Ten TB from this group were also fixed by microwave irradiation. TB were then embedded in paraffin, sectioned at 6-10μm, stained with hematoxylin & eosin, and inner ear structures were identified. Two blinded investigators then performed a histopathological analysis using light microscopy and graded structural preservation based on a subjective, multi-criterion scale: a value of “1” represented poor preservation, “2” - intermediate, and “3” - good preservation. The mean values for the four groups are as follows: immersion & microwave, n=2.57; perfusion & microwave, n=2.35; conventional immersion, n=2.33; conventional perfusion, n=2.68.

The general trend of results indicate that tissues perfused intracardially provide the best histological preservation. However, when immersion is used in place of perfusion, microwave irradiation significantly enhances histological preservation and thus may parallel conditions seen in intracardiac perfusion. We are now in the process of determining an optima concentration of fixative in microwave fixation using the above protocol. (Supported by NASA Grant NAG W1516, Depart. Funds, & NIH)


These receptors respond to low frequency μV potentials applied across the receptor epithelium. The afferents are found on the surface of a small (60 μm) diameter spherical source and (2) just-detectable level increments in on-going 50 Hz vibrations of the same source placed near the head or trunk of the fish at varying distances. At distances between 15 and 60 mm, source level at mean threshold detection (4 fish) increased with distance at an average rate of around 14 dB/distance doubling. Mean level discrimination limens (5 fish) for equivalent 10 dB sensation levels did not change with distance and were around 6.5 dB. These results show that (1) incomparable flow fields (falling off at 18 dB/distance doubling) are more likely to govern detection responses than pressure (falling off at 6 dB/distance doubling), but that (2) spatial patterns of flow amplitude, which change dramatically with distance near the source, do not affect the ability of mottled sculpin to discriminate source levels.


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589.1


In awake humans, electrical stimulation of the superior laryngeal nerve, containing mucosal afferents, elicits two types of responses in the thyroarytenoid muscles, an early ipsilateral R1 response around 17 ms and a late bilateral R2 around 60 ms. Our purpose was to examine inhibitory mechanisms controlling these muscle responses in awake humans during 1) pairs of nerve stimuli presented at rest and 2) when single stimuli are presented at rest and during volitional laryngeal tasks. With double stimulation at different inter-stimulus intervals (ISIs), conditioned R1 amplitudes decreased linearly as ISI durations were reduced from 10 s to 100 ms. Conditioned R2 responses were reduced in amplitude in relation to the time interval between stimuli but became infrequent particularly at the 500 ms ISI. Reflex responses to a single stimulus were studied during quiet respiration and three tasks: forced inspiration, effort closure and phonation. The occurrence and amplitudes of R1 responses were unaffected by task performance. R1 amplitudes were equivalent to the response amplitude in quiet added to the increased baseline activity level for a task. R2 responses, however, became infrequent during task performance in comparison with rest. The R2 response inhibition suggests that these reflexes are normally suppressed during speech. The inhibitory interval affecting the occurrence of R2 responses between 250 and 500 ms following stimulation, may contribute to the cyclic nature of repeated coughs on one exhalation. The results suggest that R1 and R2 are independently modulated.

589.2

MECHANICALLY EVOKED PERIORAL REFLEXES IN INFANTS, CHILDREN, AND ADULTS. S. M. Barlow*, D. S. Finoa, P. T. Bradford, and R. Andreotta. Department of Speech and Hearing Sciences and Program in Neuroscience, Indiana University, Bloomington, IN 47405.

Infants, children, and adults were used to investigate the effects of mechanical stimulation on perioral reflexes. It was hypothesized that the reflexes would be elicited by mechanical stimulation of the lower face. Supported by NIDCD (R01 DC 00365-05).

589.3

EFFECTS OF ANKLE POSITION ON M- AND H-WAVE AMPLITUDES IN HUMAN SOLEUS HOFFMAN REFLEX TESTING. S. C. Allison and E. L. Winter. San Diego Microscopy and Imaging Resource, Departments of Neurosciences, and Pharmacology and Anatomy, Unives. of Cal. San Diego, La Jolla, CA 92039; Department of Neurosciences, Albert Einstein College of Medicine, Bronx, NY 10461.

Ampullae of Lorenzini are lamellacentral electroreceptors sensitive to microvibrations across their sensory epithelium. Neuropil receptor cells transduce these signals and excite primary afferents via glutamatergic synapses. Tonic impulse activity in afferent fibers is increased by lumen-negative stimuli and decreased by lumen-positive ones. Clusin and Bennett (J. Gen. Physiol 73:703, 1979) concluded that apical Ca\textsuperscript{2+}-influx, induced by lumen-negative stimuli, depolarized the basal faces, activating voltage-sensitive Ca\textsuperscript{2+}-channels in them and causing transmitter release. Confocal ratio imaging of indo-1 loaded receptor cells at 0.1-15 frames/sec showed high (Ca\textsuperscript{2+})\textsubscript{i} did not spontaneously alter electrical activity. (Ca\textsuperscript{2+})\textsubscript{i} could be lowered by loading the AM-ester in Ca\textsuperscript{2+}-free EGTA medium or raised by metabolic poisons. Some cells loaded with fluo-3 via patch-clamp were tested at nine dorsiflexion and greater H-wave amplitude with ankle plantarflexion.

EFFECTS OF ANKLE POSITION ON M- AND H-WAVE AMPLITUDES IN HUMAN SOLEUS HOFFMAN REFLEX TESTING. S. C. Allison and E. L. Winter. San Diego Microscopy and Imaging Resource, Departments of Neurosciences, and Pharmacology and Anatomy, Unives. of Cal. San Diego, La Jolla, CA 92039; Department of Neurosciences, Albert Einstein College of Medicine, Bronx, NY 10461.

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589.4


This abstract reports the effects of downtraining the biceps brachii spinal stretch reflex (SSR) on the SSRs of the brachioradialis. Sixteen spinal cord injured subjects with involvement of C-5 and C-6 spinal levels have participated or are participating in the study. Subjects were randomly assigned to control (n=8) and treatment (n=8) groups. For both groups the first 6 and last 4 sessions (follow-up) involved ng operant conditioning of the biceps brachii. The middle 24 sessions involved operant conditioning for the treatment group while all sessions were the same as baseline and follow-up for the control group. The mean biceps SSR decreased from baseline during training by an average of 25.6% while the mean biceps SSR increased by 1% from baseline for control subjects. The brachioradialis SSR, which was not the target of training, decreased by an average of 32% during training and decreased an average of 15% for control subjects. The results indicate that the non-targeted brachioradialis is downtrained during the downtraining of the biceps brachii in spinal cord injured patients.

Supported by the AMERICAN PARALYSIS ASSOCIATION.
589.5 VIDEO MOTION ANALYSIS OF KNEE SWING IN SPASTIC SPINAL CORD INJURED PATIENTS BEFORE AND AFTER PERINEAL ELECTROSTIMULATION. P.W. Nance*, L.S. Halstead, S.V. Seager, Dept. Medicine, University of Maryland, Baltimore, MD 21201-1587.

Previously, we demonstrated that the knee swing in a spinal cord injured cat can generate locomotor-like EMG activity in a Frankel T6 SCI with complete transaction. In this study, we compare how EMG activity changes in that subject with a Frankel T5 SCI, who, by virtue of having EMG activity induced by an upper extremit y Jendrassik maneuver, presumably had some supraspinal input. Both probe paraplegic subjects were suspended by a hydraulic lift so that the amount of loading on the legs could be adjusted as they were manually placed through a stepping motion on a treadmill belt moving at 0.6 to 1mph. Surface EMG was recorded in the anterior, gastrocnemius, soleus, vastus lateralis, rectus femoris and hamstrings.

With training, reciprocal EMG bursts in a stepping pattern developed for each patient. The amplitude, discreetness and timing of bursts were modulated by the pattern of weight support (air stepping vs varying the level of weight supported by a leg) and speed of the treadmill belt. The activity of agonists, antagonists and synergists during different sensory conditions was best appreciated by joint probability density plots (de Gennaro, Frankel 555:202, 1991).

Rhythmic oscillation of the lower extremities enables lumbosacral neurons to organize sensory input to generate locomotor-like output when there is little or no clinical evidence of supraspinal influence.

589.6 SENSORY INPUT DURING TREADMILL TRAINING ALTERS RHYTHMIC LOCOMOTOR ELECTROMYOGRAPHIC (EMG) OUTPUT IN SUBJECTS WITH COMPLETE SPINAL CORD INJURY. R.D. Dobkin*, V.R. Edgerton, E. Fowler, Dept.s of Neurology and Physiological Sciences, UCLA, Los Angeles, CA 90024.

Recurrent probe electrostimulation (RPES) has been used in the treatment of male incontinence. The purpose of this study was to determine if RPES can be used to modulate knee swing in SCI with complete transaction. In this study, we compare how EMG activity changes in that subject with a Frankel T5 SCI, who, by virtue of having EMG activity induced by an upper extremity Jendrassik maneuver, presumably had some supraspinal input. Both probe paraplegic subjects were suspended by a hydraulic lift so that the amount of loading on the legs could be adjusted as they were manually placed through a stepping motion on a treadmill belt moving at 0.6 to 1mph. Surface EMG was recorded in the anterior, gastrocnemius, soleus, vastus lateralis, rectus femoris and hamstrings.

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SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
REFLEX FUNCTION I

Often, effects of repetitive stimulation in normal subjects are controversial due to differences in experimental conditions. The present study was aimed at elucidating the effects of repetitive cutaneous electrical stimulation on electromyographic excitability. Eleven normal subjects with no sensory deficits were used. The purpose of each session was to evaluate different parameters of stimulation such as stimulation of a single nerve trunk versus a sensory nerve (the median and the ulnar nerves) at two different intensities (50 and 99 Hz). A session where no baseline data were obtained failed to elicit a peak-to-peak amplitude of H/c as well as a small M response. Within each session, H-reflexes were recorded every 30 s for 5 min. Control values were taken for 5 min prior to (H0) and for 10 min after a 30 min cutaneous stimulation was administered at the same frequency (H1). Although no statistically significant group effects were found, there was a tendency towards a reduction (20% H0/ H1) of the H-reflex in 63% of the subjects after 30 min of repetitive stimulation at 99 Hz of either nerve. Thus, these parameters of stimulation appear to be optimal for decreasing electromyographic excitability in normal subjects. Further experiments will test for the efficacy of these TENS parameters on spasticity.

589.11 EFFECTS OF REPETITIVE CUTANEOUS ELECTRICAL STIMULATION ON EMOTONERONAL EXCITABILITY. C. Goulet, M. Levin, A.B. Arsenault*, D. Simard, B.C. Maitland, G. Goulet, Physiotherapy Program, University of Ottawa, School of Rehabilitation, University of Montreal, and Research Centre, Montreal Rehabilitation Institute, Montreal, Canada.

Spasticity is a frequent and complex sequel to central nervous system injury. The neurochemical basis for the origin of spasticity is largely unknown. Various neurotransmitters have been implicated such as -aminobutyric acid (GABA). Drugs designed to mimic GABA activity can influence the severity of spasticity but are limited by side effects. Glycine is one of the most abundant neurotransmitters in the spinal cord. It is known that glycine may modify abnormal motor behavior expressed as spasticity. Spasticity is a frequent and complex sequel to central nervous system injury. The neurochemical basis for the origin of spasticity is largely unknown. Various neurotransmitters have been implicated such as -aminobutyric acid (GABA). Drugs designed to mimic GABA activity can influence the severity of spasticity but are limited by side effects. Glycine is one of the most abundant neurotransmitters in the spinal cord. It is known that glycine may modify abnormal motor behavior expressed as spasticity.


In rabbit skeletal muscle fibers, responses similar to local twitch responses were elicited in muscle fibers of myofascial trigger point in man could be elicited by mechanical stimulation on certain "responsive bands". A myofascial trigger point needle was inserted into the most responsive site of the band repetitively at a rate of one per sec. A bipolar EMG recording needle was inserted into the other relatively "inert" end of the band. The visible LTRs and the EMG activity failed to appear after 3-15 times of repetitive needling. If the lidocaine (0.5%, 0.1-0.2 ml) was injected simultaneously during needle insertion, the visible LTRs and EMG activity disappeared with only 1-3 needle insertions. The above findings are consistent with clinical trigger point injections eliminate LTRs at that site.
CONTROL OF POSTURE AND MOVEMENT III

950.1 INFLUENCE OF AUDITORY PRECUEING ON AUTOMATIC POSTURAL RESPONSE J.W. McChesney, H. Seidler and M.H. Woolfson* Exercise and Movement Science, University of Oregon, Eugene, OR 97403.

An experiment was conducted to evaluate the influence of precueing on posture control. Two series of trials were run on each subject to assess the influence of a warning signal on automatic postural response muscle onset latency times in the gastrocnemius (G) and tibialis anterior (TA) respectively. While standing on a moveable platform, each of four subjects was exposed to an audible tone of 50ms duration that preceded a balance disturbance by 500ms. This tone was used as a prece to warn of the forthcoming balance perturbation. The perturbations were anterior and posterior translations (3cm) at 30 cm/sec. Unilateral electromyographic activity was recorded from G and TA. In the first half of the experiment (series A), a white noise tone prece as well as no prece and catch trials were utilized in 54 random trials. In the second half of the experiment (series B), 60 random trials utilizing a high/low, directionally specific tone prece, no prece, and catch trials were run.

In series A, a decrease in mean muscle onset latency time was observed in TA (94 ± 10ms to 84 ± 11ms) and G (106 ± 11ms to 95 ± 8ms) following forward and backward platform perturbations respectively. During series B, the TA and G latencies were decreased by 12% (96 ± 10ms to 84 ± 11ms) and 13% (100 ± 9ms to 86 ± 10ms) respectively. In both series, G onset was more sensitive to decrease than TA. The directionally specific tone prece proved to be more effective as a prece. We thus conclude that prior knowledge of the upcoming balance perturbation can reduce postural muscle onset latency times.


Humans prefer to use the "ankle strategy" over the "hip strategy" in response to small perturbations of the support surface (Horak et al., Exp Brain Res 82:167-177, 1990). The reasons for this preference remain largely speculative. We used a musculo-skeletal model of the human body to study the effectiveness of these control strategies in relation to stability and control constraints. Musculoskeletal actuator models for 14 muscle groups were combined with a model of the mechanics of the body to produce the dynamical equations of motion, which were used to map the set of all feasible accelerations of the body when the ankle strategy is used (i.e., only acceleration about the ankle is permitted) but not when the hip strategy is used (i.e., when ankle flexion is combined with hip extension, or vice versa). This corroborated with data showing that the ankle strategy is used in response to small disturbances, but beyond a certain size of disturbance, the hip strategy is employed.

Analysis of muscular effort indicates that the hip strategy is more efficient and faster than the ankle strategy. This leads to the question of why the ankle strategy is preferred, even for small disturbances. Results from optimal control analysis suggest that neither speed, energy, stabilization of the center of mass, nor maintenance of a stable platform for the head are considerations which explain the preference for the ankle strategy. (Supported by NIH grant NS 17662 and the Dept. of Veterans Affairs.)


Voluntary arm movements result in the application of reactive forces to the body and the alteration of the whole body centre of mass (COM) position. In this study, the effects and interactions of these mechanism on the postural response during the upright stance position were assessed. Subjects were instructed to rapidly raise their arms during four conditions which combined the polarity of the reactive forces applied to the body by the arms and the polarity of the displacement of the mass of the arms. Kinematic, kinetic and EMG analyses for each task were performed. Simulation model was developed to further examine the effects of the reactive forces. Two model conditions were considered: a) no inlination, and b) complete, instantaneous reactive forces. The maximum static arm torques (torque) of the hip, knee and ankle appeared simultaneously and were opposite in polarity to the focal moment. The hip/knee peak muscle moments in the arm strategies were lower than in the ankle strategies. This leads to the question of why the ankle strategy is preferred, even for small disturbances. Results from optimal control analysis suggest that neither speed, energy, stabilization of the center of mass, nor maintenance of a stable platform for the head are considerations which explain the preference for the ankle strategy. (Supported by NSERC and MRC)


We recorded muscle activity (EMGs) from both sides in each of five bluegill sunfish, Leporinus macrochirus, from fine wires electrodes implanted in superficial myomeric muscle at four standard locations along the length of each fish and from deep myomiic muscle at two positions. All trials were videotaped at 400 images/s, and we placed the fish in a flow tank to obtain swimming speeds of about 1.6 lengths per second. We presented 5 stimulus intervals, we dropped an object into the water to elicit startle responses (C-starts) both from fish during swimming and at a standstill. We obtained a total of 40 C-starts during swimming (6 of these occurred at higher speeds) and from a standstill (at least 9). While we were not able to determine the number of observations for each individual. The general features of the EMGs from both types of C-starts were similar, with synchronous onset of high amplitude activity from both superficial and deep muscle along the entire side of the fish that forms the concave side of the C. During steady swimming, the EMGs from the superficial muscles were unilateral and propagated posteriorly, whereas the C-starts of C-starts during swimming, the onset of large amplitude EMGs involved in C-formation occurred at any time with respect to the rhythmic pattern of low amplitude EMGs involved in steady swimming. These data suggest that the circuitry involved in the startle response can override the motor pattern generated for continuous swimming.


An appealing motor strategy for the control of arm posture and movement, suggested by E. Bizzi, N. Hogan, T. Flash and others, suggested that the brain could use a unified treatment for movement and posture control. Control of posture results from setting the co-activation levels of agonist and antagonist muscles so that a single attractive equilibrium position of the hand is coded. According to the virtual trajectory hypothesis, the ability to maintain stable postures is a building block for arm movements. The nervous system continuously vary the activities of the muscles and generates a time-sequence of attractive equilibrium positions called the virtual trajectory. The strategy is attractive if an easy to compute virtual trajectory can reproduce realistic movements (Flash, 1987). The size of the hand stiffness predicted by Flash, were much larger than those measured in humans during posture control. We proposed an alternative control for hand movement, the minimum-muscle-tension-change model. It assumes the nervous system must solve inverse dynamics for generating the muscle tensions during movements. We simulated arm movements using a 17-muscle model of the monkey’s arm, and a new algorithm which allows the non-zero muscle tensions at the beginning and end points. The simulated horizontal arm movements are quite similar to experimental findings in humans. Furthermore, our simulations predict antagonist co-activation at the initial and final (equilibrium) points. During the movement, however, the model predicts reduced co-activation of the antagonists in a coordinated manner. Therefore, the hand stiffness was reduced during movement relative to the level during posture control. This is contradictory to the requirement of the virtual trajectory hypothesis (Flash 1987). Our predictions for reduced stiffness during movement are supported by experimental data (Bennet et al, 1992). We conclude that the unified approach for the control of posture and movement, based on a simple virtual trajectory, is not supported by our findings.


Anticipatory postural adjustments were investigated in 5 patients with Parkinson’s disease (PD) and in 5 age matched control subjects. While standing on a force platform, subjects were required to rapidly raise one leg in a lateral direction to an angle of about 45°. Kinematics were measured using an ELITE system with reflective markers placed at multiple sites on the trunk and legs. Surface EMG recordings were obtained from 4 muscles in each leg. To maintain equilibrium during this task requires a number of postural adjustments which have been well characterized in young normal subjects (Mouchnino et al., J. Neurophysiol. in press). Prior to the onset of lateral displacement of the moving leg (time T2), there is a shift in the center of pressure on the force platform (offset at the time T2), initially toward the side of the moving leg and then a transfer of weight to the support leg. The mean value of T1-T2 interval was 570 ms. These force changes were accompanied by displacements of the trunk markers toward the support side. In the more severely affected patients with clinical evidence of postural instability the following abnormalities were observed: 1. the amplitude of the initial shift in the center of foot pressure was diminished. 2. The T1-T2 interval was markedly prolonged (mean value: 1331 ms). 3. the amplitude of displacement of the trunk markers toward the support side was reduced. 4. EMG recordings revealed that most muscles were tonically active and did not show bursts of activity or periods of inhibition. In summary, a T1-T2 interval was longer in the normal subjects. These results suggest that mechanisms for programming anticipatory postural adjustments are impaired in some patients with PD.
590.7

Extraretinal information influences motion perception, and motion perception influences postural control. Thus, extraretinal inputs might play a significant role in postural control, particularly if retinal inputs are minimal. We tested this idea by evaluating postural sway in 15 Parkinson patients while subjects were blindfolded and 32 young subjects for 4 fixation conditions: World-fixed visual (V) or remembered (R) target, Head-fixed visual target (H) and No target (NT) fixation. Subjects let their sway wander in the N condition. Sway measures were derived from force platform center-of-pressure outputs; eye movements were measured via AC-coupled electrooculograms (EOG). Sway was reduced for conditions V and H, but not R, vs N (p<0.05). Subjects maintained good fixation stability in V, H, and R conditions, and made larger and more frequent ascodes in the N condition (p<0.05). Better fixation was associated with improved performance on posture scores in selected conditions. Our results indicate that extraretinal inputs associated with fixation suppression of the vestibular-ocular reflex contribute to postural control, especially when retinal input is limited, and have functional implications for falls among the elderly in reduced light conditions. Supported by NASA Grant NAG 9-295.

590.8
STABILIZATION OF POSTURAL SWAY WITH HAPTIC INFORMATION. J.J. Jeka* and J.R. Lackner. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

Different forms of sensory information such as vision and touch influence the ability to maintain an upright posture. We studied the role of haptic information on the stabilization of postural sway while subjects (N=5) maintained a tandem Romberg stance on a Kistler force platform. A metal bar placed laterally beside the force platform was used to measure the amount of force applied with the right index finger during 24 s trials. Subjects were tested in three touch conditions: no support, light touch (< 100 g of pressure) or pressure support (as much force as desired) both with eyes open and eyes closed.

The results showed a significant touch x vision interaction effect (p<0.01) on mean sway amplitude (MSA). MSA with no support-eyes closed was more than twice that of any other condition. Moreover, MSA with light touch-eyes closed was significantly less than with no support-eyes open (p<0.01). Thus, touch information is more effective than vision in decreasing MSA in the tandem Romberg stance. Average applied force with pressure support (370 g) was almost ten times that of touch support (38 g). The latter force has been shown to be less than that necessary for mechanical support (Holden, Ventura & Lackner, 1987). Soc. Neurosci. Abstr., 13 (1), p. 348. Supported by NIH Grants F32-NS00025-01 and NASA Grant NAG2-515.

590.9
SEPARATE SYSTEMS FOR TONIC, TRIGGERED, AND CENTRALLY INITIATED POSTURAL CONTROL: EFFECTS OF LEVODOPA. F.Horak*, J.Frank, G.Suhpeter, M. Stephenson, B.R. Roy . Brain Research Institute and Department of Anatomy and Cell Sciences Insit. of Good Samaritan Neurological Sciences Insit. of Good Samaritan Hospital, Portland, OR 97209

Levodopa had an opposite effect on triggered postural responses and on centrally initiated postural movements in 15 parkinsonian subjects. Levodopa increased phasic muscle bursts associated with centrally initiated toe rise movements but decreased the size of phasic bursts triggered in response to surface displacements. Levodopa also reduced baseline tonic muscle activity which reduced resistance to external displacements but increased the ability to generate centrally initiated toe rise movements.

These results suggest that there are at least three separate postural systems: 1) background tonic, 2) centrally initiated, and 3) peripherally triggered. Parkinsonism affects all three postural systems. However, dopamine replacement directly improves only the tonic and centrally initiated postural systems. Supported by grants from NIA and NSERC.

590.10
Modification of Motor Evoked Potentials (MEPs) in Lower Limb Muscles by Motor Task.


Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston TX 77030

Motor unit discharge rate, and MEP amplitude vary with muscle length. However, the effects of muscle length on motor unit discharge rate and MEP amplitude are not well understood. This study examined the relationship between muscle length and motor unit discharge rate and MEP amplitude. The experimental group consisted of 10 healthy subjects, age 25-35. The subjects were tested in the quadriceps, hamstring, and tibialis anterior during isolated isometric contractions. The results showed a significant length effect on mean discharge rate (p<0.05) and a significant length effect on mean MEP amplitude (p<0.05) in all muscles. The results indicate that muscle length affects motor unit discharge rate and MEP amplitude in all muscles tested. Supported by NIH grants NS09025 and NS09026.

590.11

One method used to assess the degree of motor unit synchronization involves obtaining averages of unrectified and rectified surface EMG signals. The results showed a significant length effect on mean discharge rate (p<0.05) and a significant length effect on mean MEP amplitude (p<0.05) in all muscles tested. The results indicate that muscle length affects motor unit discharge rate and MEP amplitude in all muscles tested. Supported by NIH grants NS09025 and NS09026.

590.12

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Supported by USPHS grants AG 0900, GM 08400, NS 20544 and NS 08564.

Muscleontological behaviors are a function of kinematic variables (position and velocity), independent control variables, as well as variables characterizing the interaction between MN pools mediated by interneurons via reflex and descending pathways. The concept of muscle activation area (MAA) has been used to explain EMG patterns associated with single and multi-joint movement (Feldman et al. 1990). To test this concept, we recorded EMG signals from soleus and extensor muscles as we altered the position and velocity of unloaded deflections of the wrist arising from unloading. This instruction is assumed to be associated with an invariant control signal. Sudden unloading produced a silent period in flexor muscles and stretch reflex in extensor muscles, and the wrist stabilized with a new final position dependent on the final torque. The combinations of posture and velocity associated with the onsets and offsets of the silent periods were represented by points on the phase plane. The set of points associated with different levels of unloading produced a straight line on the phase plane diagram corresponding to the border of the MAA for given initial conditions. When experiments were repeated from an initial wrist angle of 30° flexion, the border of the MAA shifted correspondingly. The change in the initial position (X) is assumed to be associated with a change in the control signals and our results suggest that this change can be visualized as a change in the position of the border of the MAA. The EP hypothesis suggests that the position of the border may be an indirect measure of descending influence to and static y MNs, whereas its slope may be a measure of the level of dynamic activity of the system (damping coefficient).

FORCIBLE FIELDS AND MUSCLE USE STRATEGIES THAT UNDERLIE REFLEX BEHAVIOR IN THE SPINAL FROG. Simon Gostigian, Department of Brain and Cognitive Science, M.T. Cambridge, MA 02139.

Spinal frogs perform motor tasks with a complete musculomotor limb. We have suggested this ability rests on a few movement primitives residing in the spinal cord. To test this hypothesis, we explored the three-dimentional forces that underlie different reflex behaviors in measured in spinal frogs. The frog's brachial plexus was transconductor. The muscle activity and force responses were measured in the leg when the reflex behaviors were mediated by the semi-tendinosus muscle. The response was moved to a 3-D grid. The response force patterns through space were analyzed as three dimensional force fields. These force fields and the electromyographic activity were compared with the results measured during and following free limb responses to the same stimulus. The analysis showed that the spatial distribution of force fields for a particular behavior were almost constant between trials and frogs. Two qualitatively different patterns were found: squid-like and pond-like, and force control. (3) The force field patterns showed very little adjustment to alterations of body orientation to gravity. (4) While the active force patterns had fixed structures, the kinematics generated showed variability due to amplitude variations of the elicited force fields. (5) A small set of fixed muscle synergies underlie many of the movement primitives. The results support the notion that fixed muscle synergies encapsulate, but remain a matter of debate whether or not the afferent signal sent to the central nervous system accurately represents muscle force. The question is often addressed by comparing GTO afferent firing with forces produced by reflex contraction or by stimulated responses of mechanical axons. We have reinvestigated the problem by stimulating most of the soleus MUs in a distributed and rate-modulated way, to assess whether (1) single GTOs and/or (2) populations of GTOs, activated by populations of MUs, carry signals which reflect whole muscle force.

MECHANICAL PROPERTIES OF CAT SOLEUS MUSCLE ELICITED BY REPEATED RAMP STRETCHES: A SIMULATION STUDY. C. Lin* and W.Z. Rymer, Departments of Biomedical Engineering and Physiology, Northwestern University, Chicago, IL 60611.

The objective of this study was to examine the intrinsic mechanical properties of muscle during constant velocity ramp-stretches of sufficient amplitude to induce muscle yield, with the goal of establishing the relative magnitude and time-dependence of viscous and elastic components of the mechanical response. The soleus muscle was a deafferented decerebrate cat preparation was activated by a crossed-extension stimulus. Two experimental paradigms were employed on a total of five cats. The first consisted of applying two ramps (each 2-mm amplitude), separated by a variable time interval. The velocities of the two ramps were usually the same and ranged from 10 to 30 mm/s. The principal results revealed that resistance, velocity, and muscle force response were sigmoidal in form. Force response after yield remained constant until the end of the ramp, and the force level depended solely on the rate of velocity, not on previous mechanical events, similar to a viscous damper. However, the force response recorded prior to the steady-state response was history dependent. Specifically, stiffness measurements taken shortly after the onset of the stimulus were higher than those sampled at the end of the ramp. (1) The recovery of the initial stiffness was very fast to primarily elastic behavior.

EFFECTS OF MUSCLE AND MUSCLE FIBER ABNORMALITIES ON THE SIMULATION OF A MODEL OF CAT MEDIAL GASTROCNEMIUS MUSCLE. J.L. Weytjens and U. Windhorst. Dept. of Clinical Neurosciences, University of Calgary, Alberta T2N 4N1, Canada.

The results presented previously. Each motoneuron is made to fire with interpulse intervals randomly distributed around its central interval, and the neurons of the pool are asynchronous. The EMG response is history dependent on the stimulus. It is clear that this history dependence could be modulated by the time delay of the RC synaptic potential, and the time delay of synaptic potentials at the synaptic terminals of RCs. We have simulated a model of the motoneuron-pool and its muscle. This model is based on the available physiological data regarding rate-dependent properties of motoneurons and muscle fibers, primarily from studies of the rat and cat medius gastrocnemius muscle.

We have added an EMG component to an earlier version of this model, presented previously. Each motoneuron is made to fire with interpulse intervals randomly distributed around its central interval, and the neurons of the pool are asynchronous. The EMG response is history dependent on the stimulus. It is clear that this history dependence could be modulated by the time delay of the RC synaptic potential, and the time delay of synaptic potentials at the synaptic terminals of RCs. We have simulated a model of the motoneuron-pool and its muscle. This model is based on the available physiological data regarding rate-dependent properties of motoneurons and muscle fibers, primarily from studies of the rat and cat medius gastrocnemius muscle.

To study the effects of increasing the Renshaw cell (RC) synaptic strength on motoneuron firing, an RC-motoneuron pool was simulated using the MacGregor point neuron model. Parameters for RCs and motoneurons were generated by matching published input resistance, time constants, and current-rate data. Spontaneous strengths of motoneurons on RCs were set to be sufficient to cause bursting firing of the neurons. The neurons were arranged on a rectangular grid, and the effects of synapses were spatially limited to one row or column. The synaptic current on the motoneuron from each RC terminal was 0.11 A. The average number of terminals from each RC to each motoneuron was increased, to enlarge both the magnitude and the statistical variation of ISPs on the motoneurons. This allowed a comparison of effects at the level of increasing RC synaptic strength without introducing extra synchrony. The RC-motoneuron pool was simulated with constant current level. Firing synchrony was measured as the coefficient of variation (c.v.) of the total activity of the motoneuron pool; it was assumed that RC-mediated desynchronization would reduce variability in the input to the motoneuron. It was found that the c.v. fell off slowly with the number of terminals until it reached a plateau, then began a shallow rise. This implies that the small magnitude of RC effects is actually optimal for desynchronization of motor pool.

This work was supported by NIH grant NS 28076-02 and NS2925-01.

EFFECTS OF MUSCLE AND MUSCLE FIBER ABNORMALITIES ON THE FORCE-EMG RELATIONSHIP IN A MODEL OF CAT MEDIAL GASTROCNEMIUS MUSCLE. J.L. Weytjens and U. Windhorst. Dept. of Clinical Neurosciences, University of Calgary, Alberta T2N 4N1, Canada.

This work was supported by NIH grant NS 19331.
MUSCULOSKELETAL KINEMATICS DURING CONTROLLED HEAD MOVEMENTS IN CATS. E.A. Keshner*, S.D. Garimella, J. Hanson, and B. W. Peterson. Dept. of Physical Therapy, Univ. of IL at Chicago and Dept. of Physiologic, Northwestern Univ. Med. School, Chicago, IL 60611. Supported by grants BNS907055 and N2S2490.

Patterns of muscle activation during voluntary head movements in the cat were consistent within each animal, but varied between animals producing the same head movement (Keshner et al., Exp Br Res, 1992). One source of this relative arrangement of the cervical vertebrae during the head movement. In this study we recorded simultaneous video-flouroscopic and neck muscle (biventer, BIV, complexus (CPX), occipitoscapularis (OCC), and splenius (SPL)) EMG data from a cat performing 17° sinusoidal (0.25 Hz) head tracking movements in an initial plane in order to identify the relations between intervertebral actions and muscle activation. Video-opaque markers were inserted into the anterior/posterior and lateral aspects of C1-C7 to measure vertebral displacement every 300 ms during 20 sec of sinusoidal head movement. Vertebral movement phase led stimulus position by as much as 45°, and the extent of motion between vertebrae was unequal. CPX exhibited 11.5° more motion than the C2, which moved not less than 0.5° more than C3. The differential motion between C1 and C7 was about 1.°. Unlike the vertebrae, the head moved in phase with stimulus position, and exhibited the greatest displacement, moving as much as 5° more than C1. BIV phase led head extension. CPX and OCC either phase led peak flexion of the head, or peaked in phase with peak downward flexion of the head. The latter behavior was accompanied by small shifts in C7 and C5 toward a greater phase lead re peak head extension. SPL exhibited the greatest response peaks, leading both peak extension and peak flexion of the head. The overall picture suggests a whiplike motion of the head and neck where the movement of the intervertebral properties of the head are used to produce a position matched response.


This study examined the biomechanics of stance in the cat at various fore-hind interlimb distances. As stance distance narrows, the freely standing cat can either: 1. maintain a horizontal trunk and change the limb inclination; 2. constrain limb inclination and arch the trunk; C. stand quietly on 4 force plates mounted at fore-hind distances of 33, 30, 25 and 20 cm. The 3D ground reaction forces and kinematics were recorded. Joint moments were computed for the left hind limb.

At 33 cm, the reaction force vector for the hind limb (line from toe to hip) were both inclined forwards and inwards. At stance distance decreased, the force vector inclination gradually decreased to become vertical at 20 cm. The limb axis inclination decreased more rapidly, becoming vertical at 30 cm and backwardly directed at 20 cm. Concurrently, the sagittal ankle moment decreased while hip and ankle moments increased. However, the sum of absolute 3D moments at the hip, knee, ankle and first tarsus-phalangeal joints remained similar. The spine joining the shoulder and the hip remained constant in length and orientationally. Thus, in varying stance distance, cats use the first strategy of controlling limb incline and second strategy of controlling the size of the soleus H-reflex (Natural Reciprocal Inhibition, NRI) in normal humans. This occurs during locomotion. TA contractions producing either constant (static) force or dynamic force changes. We measured NRI in patients with partial spinal cord injury (SCI), and compared these measurements with those from normal subjects.

Subjects were seated with their foot strapped to a metal plate which measured force during repetitive kinematic and dynamic contractions. At force levels which were comparable during the ramp and during static contractions, the tibial nerve was stimulated to reflex recruitment curves were obtained during both static and dynamic forces were compared by absolute torque levels and percentages of maximal voluntary contraction.

In normals, TA contraction produced NRI during both static and dynamic contractions. NRI was demonstrated at all test reflex amplitudes, even in normal subjects with large resting H-reflex. In contrast, partial SCI patients demonstrated a reduction or loss of NRI. Impaired NRI was especially evident when dynamic contractions were compared. Interference with mechanisms producing NRI may contribute to the clinically observed motor deficits in these patients.

Simple pre-planned movements can be defined as realizations of an original central pattern transformed by a parametric transform. The values of parameters are established by the motor control system prior to the movement. As a first approximation, under this transform, we take scaling in space and time. Assume that the original pattern is deterministic and does not contribute to movement variability, and the parameters are random. Thus, the variance of trajectories (Var(t)), velocity (Var(v(t))), and acceleration (Var(a(t))) can be expressed as weighted sums of two basic functions by variate:

$$\text{Var}(t) = \alpha t^2 + \beta$$

where $$\alpha$$ and $$\beta$$ are variances of established space and time constant, respectively. Note that in the expressions, the first and the second terms are, correspondingly, the spatial and the temporal components of the variance.

591.7 EFFECTS OF PRACTICE AND COMPLEXITY ON HUMAN MOTOR LEARNING IN A CONTINUOUS TASK. Hanneke van Mier*, Wouter Hulstijn (2), Steven E. Petersen (1). (1) = Department of Neuroscience, University of Amsterdam, The Netherlands. (2) = Department of Experimental Psychology, University of Leiden, The Netherlands.

Large differences are found between the planning of observed movements patterns, like letters, and that of novel, unpracticed movement patterns. The difference stimulated a study in which changes in the planning of a movement were studied as a function of practice. The planning, in which a novel movement pattern transforms into a well-learned movement pattern, was studied in a maze drawing task. Twelve subjects learned to move a pen through cut-out maze patterns with their non-dominant hand. Maze patterns consisted of six, eight, ten, or twelve segments that were connected by intersections. Total path length of each maze was 24 cm. Although the mazes could be traced continuously in a clockwise direction, selecting a wrong turn at an intersection led to a dead end. Performance at execution of earlier segments. Finally, the results suggest that this learning process proceeded through qualitatively different learning phases.


Glycine is recognized as a mediator in postemtpnic inhibition in the spinal cord. Its depletion has been linked to rigidity after ischaemic damage in experimental animals. The concentration of Glycine in CSF of 10 spastic and 10 non spastic age matched children was determined. The spastic children had antecedents of perinatal asphyxia. The values of glycine were measured by HPLC, using a W a ters 510 equipment with fluororesent detector. The amaxinaeoycids were separated with a 15 cm. long, Nova Pack C 18 column. A gradient with two solvent was used, solvent A was a phos-phate buffer (pH 7), solvent B was 40% of the buffer and 60% of tetrohydrofuran. The fluorescent intensity was measured at an excitation wavelength of 425 nm. The values of glycine was identified by comparison with the retention time of the authentic compound. Glycine was quantified by the measurement of the peak area under the fluorescence- line receptor intensite. Water 240. The values detected (mean 2.3 umol S.D. 0.83), in the spastic group were significantly lower (P<0.001) than those observed in the non spastic children (mean 6.6 umol S.D. 1.14). It could be suggested that low CSF glycine reflects loss of inhibitory Renshaw cells. This imbalance could be in part responsible for spasticity.
CONTROL OF POSTURE AND MOVEMENT IV
THURSDAY PM

591.11
NEURONS OF THE ANTERIOR CINGULATE MOTOR AREA ARE RELATED TO VOCALIZATION AND OTHER OROMOTOR BEHAVIORS. R. West* and C.L. Larsson. Dept. Of Communication Sciences and Disorders, Northwestern University, Evanston, IL 60208.

A large number of anatomical, stimulation and lesion studies have indicated the anterior cingulate motor area (ACMA) may play a role in vocal behavior. To further explore this possibility we have recorded neural activity from what we believe is the area of the cingulate sulcus during self-paced vocalization, the awake monkey (Macaca nemestrina). We also recorded activity while the monkey performed a self-paced jaw opening task to determine whether related units were present. The majority of related neurons modulated their activity in the same manner during both tasks, but with different latencies to behavior onset. Vocalization related neurons often had long lead times, and these generally became short lead times during the jaw task. Some cells related to both tasks, however, had drastically different firing patterns. Thus far, a small number of cells related specifically to vocalization area, the cell related specifically to jaw opening have been observed. We have also observed cells involved in a variety of other oromotor movements, such as tongue protrusion, lip rounding, and jaw movement. Time of light (flashed or go signal) prior to the delay. The jaw exercise induced a significant amount of excitation in the low activity thresholds of the skin studied in human subjects. Exercise consisted of brief (10-15 s) contractions of jaw closing muscles, flexions of the hand or foot against varying loads (10-30% of the maximum load). A light flash indicated the start and the end of the exercise. The hand exercise produced a load-dependent and rapidly attenuating excitatory effect in the exercising hand but not contralaterally. The foot exercise at comparable intensity produced a smaller effect. The threshold elevation was significant also immediately prior to EMG responses of the arm but not prior to 20 s responses of the jaw closing muscles. This threshold elevation was attenuated already before the end of the shortest exercise period (1 s). Thus, isometric exercise produces a rapid, suddenly attenuating threshold elevation to electrotactile stimulus 0.1 s. A plausible explanation for the threshold increase is the suppression of afferent input due to barreage from motor to sensory areas of the brain.

591.13
MODULATION OF CUTANEOUS SENSITIVITY BY ISOMETRIC JAW versus LIMB EXERCISE IN HUMANS. P. Kemppinen*, Y. H. Lenggenhag and A. Pertovaara. Dept. of Prosthetic Dent. and Dept. of Physiology, Univ. of Helsinki, Helsinki, Finland.

The effect of isometric exercise on electrotactile thresholds of the skin studied in human subjects. Exercise consisted of brief (10-15 s) contractions of jaw closing muscles, flexions of the hand or foot against varying loads (10-30% of the maximum load). A light flash indicated the start and the end of the exercise. The hand exercise produced a load-dependent and rapidly attenuating excitatory effect in the exercising hand but not contralaterally. The foot exercise at comparable intensity produced a smaller effect. The threshold elevation was significant also immediately prior to EMG responses of the arm but not prior to 20 s responses of the jaw closing muscles. This threshold elevation was attenuated already before the end of the shortest exercise period (1 s). Thus, isometric exercise produces a rapid, suddenly attenuating threshold elevation to electrotactile stimulus 0.1 s. A plausible explanation for the threshold increase is the suppression of afferent input due to barreage from motor to sensory areas of the brain.

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591.14
CORTICOSPINAL REORGANIZATION AFTER SPINAL CORD INJURY (SCI) IN MAN. D. Hopkins-Kosellek and R. Brouwer*. Dept. of Rehab. Therapy, Queen's Health Pvt. H. 2N 3G.

It has been suggested that the nature human central nervous system (CNS) has the ability to reorganize following traumatic injury (Lely et al. Brain Research 510:130,1990).

This study used focal transcranial magnetic stimulation (Cadwell Laboratories) from the motor cortical representation and the activation threshold of each of the hlip广大 muscles and tibialis anterior of 10 SCI subjects with low cervical lesions of greater than three years duration (n=7) and healthy controls (n=3). Two stimuli were delivered to the scalp at specific points desynchronized with (150 s) and every 4-5 s, respectively. The mean relative amplitudes of the short latency CMAs (normalized to the maximum N were elicited by supramaximal electrical stimulation of the muscle nerve) were similar between both groups for all muscles (p > 0.10). These findings do not support the ability of the corticospinal tract to reorganize following longstanding SCI.

591.15
DESCENDING PROJECTIONS OF THE MESENCEPHALIC LOMOTOR REGION (MLR) BASED UPON TREADMILL-INDUCED C-FOS PROTEIN AND ANTEROGRADE TRACT TRACING. A. Shamsa and C.A. Lobbins. S. Prilusky, L.M. Jordan* and D.M. Nagele. Departments of Pathology and Physiology, University of Manitoba, Winnipeg, MB, R3E 0W3, Canada.

The MLR is operationally defined as the area in the mesopontine region in which low levels of stimulus stimulation occur in a treadmill. However, variability in effective stimulus sites has hindered the detailed analysis of the projections from the MLR. We report here that exercise on a treadmill induces c-fos protein in the mesencephalal region of the rat, and we have found that a number of c-fos labeled neurons are produced by locomotion. The speed of the treadmill was increased from 0-0.25 m/sec and maintained for 60 min. After perfusion, brain sections were incubated with antibodies to c-fos protein and visualized via the PAP technique. The same or alternate sections were then processed for the labeling of the cuneiform nucleus. The same or alternate sections were then processed for the labeling of the cuneiform nucleus. The same or alternate sections were then processed for the labeling of the cuneiform nucleus. The same or alternate sections were then processed for the labeling of the cuneiform nucleus. This preparation has the advantage that the cuneiform nucleus is a functional part of the locomotor pathway. Supported by NIH, NIDCD DC00208.

591.12
DEDO SHAPE OR SURFACE AREA INFLUENCE PREHENSION. P.L. Weir* and C.L. MacKenzie. Dept. of Kinesiology, University of Windsor, Ontario, N9B 3P4, and Simon Fraser University, Canada, V5A 1S6.

MacKenzie & Weir (1991) have shown that when the available contacting surface area is identical between a dowel with a flat gripping surface (force dowel) and one with a curved surface (cylindrical dowel), the actual area contacted is substantially lower on the cylindrical dowel. This difference between dowels is also reflected in the activation threshold of motor cortical representation and the activation threshold of each of the hlip广大 muscles and tibialis anterior of 10 SCI subjects with low cervical lesions of greater than three years duration (n=7) and healthy controls (n=3). Two stimuli were delivered to the scalp at specific points desynchronized with (150 s) and every 4-5 s, respectively. The mean relative amplitudes of the short latency CMAs (normalized to the maximum N were elicited by supramaximal electrical stimulation of the muscle nerve) were similar between both groups for all muscles (p > 0.10). These findings do not support the ability of the corticospinal tract to reorganize following longstanding SCI.

This study used focal transcranial magnetic stimulation (Cadwell Laboratories) from the motor cortical representation and the activation threshold of each of the hlip广大 muscles and tibialis anterior of 10 SCI subjects with low cervical lesions of greater than three years duration (n=7) and healthy controls (n=3). Two stimuli were delivered to the scalp at specific points desynchronized with (150 s) and every 4-5 s, respectively. The mean relative amplitudes of the short latency CMAs (normalized to the maximum N were elicited by supramaximal electrical stimulation of the muscle nerve) were similar between both groups for all muscles (p > 0.10). These findings do not support the ability of the corticospinal tract to reorganize following longstanding SCI.
EVIDENCE FOR CHANGES IN MUSCLE MECHANICAL PROPERTIES AND STRETCH REFLEX CHARACTERISTICS IN SPASTIC HEMIPARETIC STROKE. W.D. Crews, J.P.A. Derald, J.R. McGunigal, T.C. Backhouse and W.Z. Byrnes. Sensory Motor Rehabilitation Program, Rush-Presbyterian-St. Luke's Medical Center and Dept. of Physical Medicine and Rehabilitation, Northwestern University Medical School, Chicago, IL. The primary goals of this study were to quantify joint impedance in spastic limbs of hemiparetic stroke subjects, and to identify the relative contributions from passive and reflex components of spastic muscle. Slow ramp extensions of the elbow, stretching with constant velocity, was performed on spastic upper limb joints (n=6), and with a normal control group (n=4). The passive elastic and viscous stiffness estimates averaged the torque response recorded in the absence of any significant stretch or stretch reflex EMG activity. A comparison of spastic and normal subjects revealed significant increases in passive elastic stiffness in the implicated limb relative to the non-affected limb (P<0.05). The increased stiffness was due to the specific algorithm used to map target location into the hand to remembered target locations in 3D space, they make substantial errors in matching target distance, but not in matching target direction (azimuth and elevation). They hypothesized that these errors are due to the specific algorithm used to map target location into the angular coordinates of the arm joints. Object location is essentially a static task, the trajectory of the arm being apparently inconsequential to the performance. We investigated kinesthetic perception of 3D orientation by asking subjects to match the remembered azimuth and elevation of a test bar they had previously explored with their hand. Subjects made errors in both dimensions, undershooting the target outside a linear range of ± 30°. In addition, a variable amount of offset in matching angle was present dependent on the position of the subject relative to the origin of rotation of the bar. The slope of the regression between matching angles and target angles was affected by experimental manipulation of head-centered references, as obtained by asking the subjects to rotate their head in the horizontal plane or by experimental manipulation of head-centred references, as obtained by vibrating their neck muscles at 100 Hz thereby inducing an illusory shift of subjective straight-ahead. None of these errors were observed in control experiments in which the target was presented visually to the subjects. The postulated transformation from the world coordinates of the target into the arm coordinates might then undergo an intermediate cranio-tropic stage.

CHOLINERGIC EFFECTS ON THE CRAYFISH SWIMMERET CPG. Gills Brown* and R. Mulloney. Department of Zoology, University of California, Davis, CA. There are two reasons for examining the effects of cholinergic agonists on the crayfish swimmeret CPG: 1) many primary sensory afferents are cholinergic and project to the swimmeret neuromuscular system, 2) cholinergic (muscimol) agents elicit rhythmic activity in other crustacean nervous systems (walking in crayfish, stomatogastric ganglion in the lobster). The present study examines the effects of nicotine (nicotinic agonist), pilocarpine (muscimol agonist), and carbaclo (nicotinic agonist). Can they elicit the rhythm in an inactive preparation, and how do they modulate the frequency and pattern of activity produced by proctolin? Motor neuron discharge was monitored extracellularly with pin electrodes on N₂ neurons, drugs were applied to the bath solution. Pilocarpine elicited rhythmic activity in inactive preparations with a threshold between 1 and 10 μM. Burst frequency increased with concentration up to 100 μM. When pilocarpine was applied to a preparation already activated by proctolin the burst frequency was slightly reduced. Nicotine normally failed to elicit the rhythm in an inactive preparation, but when applied to a preparation activated by proctolin, nicotine increased the burst frequency considerably. Carbaclo reliably elicited the rhythm in inactive preparations and also modulated the burst frequency in proctolin activated preparations.

The results suggest that cholinergic transmission is used in two ways: as a component of the central circuitry via muscarinic receptors, and as a mediator for the input from primary sensory afferents via nicotinic receptors.
INVERTEBRATE MOTOR FUNCTION

THURSDAY PM

592.3 MOTOR NEURONS OF THE SWIMMERET SYSTEM: MEMBRANE PROPERTIES OF ANTAGONISTS. C.M. Smith and B. Mulloney. Department of Zoology, University of California, Davis, CA 95616.

The musculature of a single crayfish swimmeret is innervated by about 69 motor neurons. When an isolated nerve cord is generating the swimmeret motor pattern, only a few of these motor neurons fire action potentials; the membrane potentials of the others do not display spiking activity. These motor neurons may represent pools of similar neurons, which are recruited in order of increasing size by stronger inputs from the central pattern generator to increase the strength of contraction of the swimmeret muscles. Alternatively, these motor neurons may possess different properties and have different functions in the swimmeret motor output.

We have recorded extracellularly from the neuropil or cell bodies of swimmeret motor neurons and recorded their resting membrane potentials, input resistances, and membrane time constants, their active responses to depolarizing current injection, and their effects on the output of the motor programs. We have also looked for feedback from these motor neurons onto the central pattern-generating network.

Most of the motor neurons appear to have similar membrane properties and activities in the swimmeret rhythm. No systematic differences were found between power-stroke and return-stroke neurons, excitatory and inhibitory neurons, or spiking and silent motor neurons with regard to passive membrane properties. Most of these neurons also displayed sustained, tonic firing that did not reset the rhythm when they were depolarized by current injection.

A small number of swimmeret motor neurons display a different electrical profile. These fired phasically with current injection; the membrane fires several action potentials with the onset of depolarization, and then settles to a steady-state depolarization that persists for the duration of the current injection.


Recordings from frontal nerve motorneurons (FNMs), supplying neck rotator muscles, demonstrate their directionally selective activation by ipsilateral wing motion and inactive inhibition by stimuli contralaterally. Recordings from premotor descending neurons (DNs) supplying neck motor neuron also reveal selective inhibition by wide-field stimuli; these DNs give rise to specific dendritic branches that extend into an area of neuropil in the lateral deutocerebrum receiving terminals of certain motion-sensitive wide-field neurons originating contralaterally in the lobula plate. The functional significance of such tangential neurons is suggested by immunocytochemistry using an antibody raised against the inhibitory transmitter y-amino-butyric acid (GABA). 20-25 immunopositive axons extend from the lobula plate, of which at least 8 match Lucifer yellow filled neurons terminating in this deutocerebral area. The relevance of GABAergic pathways to inhibition of frontal nerve motor neurons has been studied using microlesions made while recording from FNMs. Lesions across GABAergic pathways from the lobula plate first abolish the inhibitory component of the FNM response which then returns after minutes. Lesions across ipsilateral DNs abolish the excitatory FNM response which also then recovers. These results suggest distributed routes of pathways controlling head movements.

Neurotubulin and covalent fitts into heterolateral ascending and descending neuronal terminals, further supports this hypothesis. Supported by NIH Grant No. R01 EYO-7151.

592.7 ANATOMICAL AND PHYSIOLOGICAL ANALYSIS OF A RAPID STEERING MUSCLE OF THE BLOWFLY. M.S. Tu, and M.H. Dickinson. Department of Biology and Anatomy, University of Chicago, 1025 E. 57th St., Chicago, IL 60637.

In addition to the large, stretch-activated power muscles, flies possess a set of 17 direct synchronous muscles that are responsible for elaborate turning maneuvers during flight. Of the steering muscles that have been recorded, The first Basalar (B1) stands out due to its ability to fire each and every wing beat, even at frequencies exceeding 200 Hz, making it among the fastest known synchronous muscles. Its unique physiology makes the B1 a prime candidate for studying the control of wing protraction during flight. We have been using the anatomy and physiology of this muscle in Calisto as a model to elucidate the role of motorneurons in the control of steering maneuvers. We have identified B1-specific motorneurons Using microlesions developed permit an analysis of the impact of removal of B1 on the steering capacity of the blowfly. We have used this model system to study the role of B1 in the generation of forward steering maneuvers.

The central and peripheral morphology of the B1 motor neuron has been determined using low molecular weight dextrans. Both the cell body and the most extensive arborizations are ipsilateral, although one branch of the dendrite crosses the midline contralaterally. The peripheral morphology of the B1 motor neuron is much more striking: the 250 μm arbor branches into a large arborous network that innervates the entire muscle. The axon size and terminal geometry are consistent with the role of B1 in the generation of forward steering maneuvers.

Repetitive stimulation of the B1 motor axon at wing beat frequency results in tetanus, although discrete isometric force transients can be resolved at frequencies as high as 100 Hz. However, during flight, the B1 muscle is subjected to cyclic length changes at wing beat frequency. Therefore, we are currently investigating the effects of stimulus phase on the mechanical output during imposed sinusoidal length changes that mimic those which occur naturally during flight.

592.8 A NECESSARY ROLE FOR PROCTOLIN IN MAINTAINING TENSION PRODUCTION BY AN INSECT MUSCLE. J.J. Belanger* and J. Orchard, 'Arizona Res Lab Div of Neurobiology, U of Arizona, Tucson, AZ 85721, USA and 2Dept of Zoology, U of Toronto, Toronto, ON, M5S 1A1, Canada.

We have used the neuromuscular substrate underlying oviposition digging in the locust (Locusta migratoria) as a model system to try to understand the role(s) played by neurotransmitters in the control of insect muscle. The ventral opener muscle is the largest of the ovipositor muscles and produces most of the force used to dig the oviposition hole. We have previously reported the presence and some actions of proctolin in this neuromuscular system (Belanger and Orchard 1988, Soc. Neurosci. Abs. 14:248). Here, we report that proctolin is released by electrical stimulation of nerves and during normal activity of the oviposition digging system, and that this release appears to be necessary for the production of normal amounts of tension by the muscle.

Stimulation of some, but not all, units in the nerve supplying the opener muscle produces a frequency-dependent release of proctolin. Five min of 30 Hz stimulation releases approximately 8% of the total proctolin store of the muscle. During oviposition digging, the opener muscle is driven by a central pattern generator which is located in the terminal abdominal ganglion. In vitro ganglion-muscle preparations which are expressing the digging rhythm, release of about 25% of the muscle proctolin store occurs during the first five minutes of activity. This level declines to below detectability over a period of approximately 20 min. There is a concomitant decrease in the force generated by these muscles. However, when the patterned neural activity is still present and EMGs can still be recorded, implying that conventional synaptic function is still present. Adding exogenous proctolin (10⁻⁹ M) to the superfuse restores the contractions to their original magnitude.

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In a study, we examined whether there are similar differences in the claw closer muscle were dissected free. Mitochondria were visualized with mitochondrial content of the phasic and tonic motor axons. After volume of axoplasm and a greater cross-sectional area of individual area was determined using TEM. Results indicate that there are differences in mitochondrial volume in the motor axons which are similar to those mitochondria in the tonic axon.

D.A.Tamarkin* & R.B.Levine. ARL, Div of Neurobiology, Univ of Arizona, Tucson, AZ 85721.

During insect metamorphosis, neural circuits undergo dramatic reorganization that contributes to the expression of new behavior. We are investigating this reorganization by examining an abdominal proprioceptive circuit during insect metamorphosis in the hawkmoth, Manduca sexta. The effect of activity of a stretch receptor organ (SRO) on intersegmental muscle motoneurons (MN) was characterized in both the larval and adult stages. The SROs are bilaterally-paired muscle receptor organs that run longitudinally across each abdominal segment. Populations of synergistic and antagonistic MNs are described based on whether contraction of their target muscles would result in excitation of both the synergists and antagonists. These MNs differ in their physiological properties, transmitter phenotypes and structures from both segments regressed similarly such that they were significantly reduced by day P0. Therefore, none of the features that we examined were correlated with the subsequent segment-specific death or survival of homologous motoneurons.

Supported by NIH grants HD07244 and NS22508.

SEGMENT-SPECIFIC FATE OF HOMOLOGOUS MOTONEURONS IN MANDUCA SEXTA IS UNCORRELATeD WITH DEVELOPMENTAL CHANGES IN MOTONEURON ANATOMY AND PHYSIOLOGY.

L.C. Sperelakis, D.J. Sandercock, & C. Weeks. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Morphogenesis of the tobacco hornworm, Manduca sexta, is accompanied by the disorganizing of neural circuits involved in stage-specific behaviors. The loss of the proleg withdrawal reflex at the larval-pupal transformation is associated with diencephalic progression of proleg retractor motoneurons, a weakening of afferent input to these motoneurons, and the programmed death of a stereotyped subset of the motoneurons. For instance, the APR motoneurons, which innervate a proleg retractor muscle, undergo programmed cell death on the second day of pupal life (day P2) in abdominal segments A5 and A6, whereas the APRs in A3 and A4 survive and are respected in the adult. We examined the possibility that these differences in cell fate are correlated with anatomical and physiological changes by comparing the properties of APR motoneurons in segments A3 and A6 during the larval-pupal transformation.

On the third day of the final larval instar (day L3), the structure of APR's dactylic arbor was similar in A3 and A6. Prior to pupation, the dactylic arbor of APRs from both segments regressed similarly such that they were significantly reduced by day P0. There were also no segment-specific changes in the passive electrical properties of the APRs. Electrical stimulation of the nerve containing sensory afferents that synapse upon the APRs evoked compound EPSPs that were of similar mean size in A3 and A6 on day L3. A developmental reduction in the magnitude of the evoked EPSPs was quantitatively similar from both segments on day P0. Therefore, none of the features that we examined were correlated with the subsequent segment-specific death or survival of homologous motoneurons.

Supported by NIH grants HD07244 and NS22508.

THURSDAY PM

INVERTEBRATE MOTOR FUNCTION

LOCAL CALCIUM SPIKES IN THE DENDRITES OF NONSPIKING LOCAL INTERNEURONS IN THE LOCUST. Gilles Laurent*. Biology Div., 139-74, California Institute of Technology, Pasadena CA 91125.

Nonspiking local interneurons directly synapse onto pools of leg motor neurons and are thus well suited to organize leg movement and posture. During a centrally generated rhythm and in the absence of any sensory feedback, their membrane potential undergoes large polarizations (30mV peak-to-peak) centered on a resting potential of ca. -58mV. Depolarization from -60mV leads to outward rectification, which in turn leads to significant changes in membrane time and space constants. Depolarization to -40mV, however, also evokes, in 20% of dendritic morphologies, calcium spikes which can take 2 main forms. The first are fast potential oscillations (10-15mV p-p) which only occur within a small window of membrane voltages. The second are 30mV-long TTX-resistant action potentials. Whole-cell patch-clamp experiments on cultured nonspiking interneurons indicate the existence of a low-threshold transient Ca**-current analogous to Type currents described in other neurons. These action potentials could be evoked on rebound from IPSP evoked by presynaptic spiking local interneurons. Interestingly, however, such an active response can often be evoked by one only of several IPSPs which the nonspiking interneuron receives from different presynaptic interneurons. This suggests that the channels underlying these action potentials may only be expressed or functional in some, but not all, dendrites of a nonspiking interneuron. Supported by NIH and McKnight, Searle & Sloan Foundations.


During insect metamorphosis, neural circuits undergo dramatic reorganization that contributes to the expression of new behavior. We are investigating this reorganization by examining an abdominal proprioceptive circuit during metamorphosis in the hawkmoth, Manduca sexta. The effect of activity of a stretch receptor organ (SRO) on intersegmental muscle motoneurons (MN) was characterized in both the larval and adult stages. The SROs are bilaterally-paired muscle receptor organs that run longitudinally across each abdominal segment. Populations of synergistic and antagonistic MNs are described based on whether contraction of their target muscles would result in excitation of both the synergists and antagonists. These MNs differ in their physiological properties, transmitter phenotypes and structures from both segments regressed similarly such that they were significantly reduced by day P0. Therefore, none of the features that we examined were correlated with the subsequent segment-specific death or survival of homologous motoneurons.

Supported by NIH grants HD07244 and NS22508.


Axoplasmic transport of mitochondria was examined in the phasic and tonic axons using DIC microscopy, drawn with a camera lucida attachment, and their lengths were measured on a digitizing pad. Individual mitochondrial cross-sectional area was determined using TEM. Results indicate that there are differences in mitochondrial volume in the motor axons which are similar to those previously reported for the terminals. The mitochondrial volume per unit volume of axoplasm is 5-fold greater in tonic muscle axons than in phasic motor axons. This difference results from more mitochondria per unit volume of axoplasm and a greater cross-sectional area of individual mitochondrial pools. Axoplasmic transport of mitochondria was examined in the phasic and tonic axons using video enhanced-contrast DIC microscopy. Consistent with the results from the larval stage, we found that the density of mitochondrial transport in tonic axons was twice that found in the phasic axons.

In order to determine if the differences in mitochondria content and transport are activity-dependent, we have begun to examine tonically stimulated phasic axons. Preliminary results from mitochondrial measurements in fixed axons indicate that tonic stimulation of a phasic axon results in an increase in mitochondrial content. (Supported by NSF grant BNS-9121757.)


The physiological properties, transmitter phenotypes and structures of individual neurons are unique, ultimately depending on their differential gene expression. Recently, a method has been developed that allows monitoring of gene expression in single identified neurons following intracellular recording (1).

The pool of mRNA from a single neuron is amplified up to a million fold via cDNA-synthesis within the cytoplasm of the neuron, and the use of the T7-RNA polymerase reaction on the extracted cDNAs. Amplified messages are analysed using expression profiles that enable monitoring of several genes at the same time. In preliminary experiments we have tested the method in the CNS of Homarus americanus on large GABA-containing inhibitory motoneurons in the 2nd abdominal ganglion, serotonin-containing serotonincontaining neurons in the 1st abdominal ganglion and octopamine-containing crotch cells in thoracic ganglia. In all cases successful amplifications have been performed. In expression profiles amplified RNA hybridizes with vertebrate-probes corresponding to neurofilament proteins, c-fos, c-jun, GABA-receptors, glycine-receptors, K+ Na* Ca**-channels, protein kinase C, and tryptophan hydroxylase. In the long run we plan to study gene expression in single cells in differing developmental and behavioral states. (1) Van Gelder et al. 1990, PNAS 87: 1603-1667. Supported by NIH and DGf Schn 386/1-1.

ARE THERE FEEDBACK SUBGROUPS WITHIN CRAYFISH MOTOR POOL? Peter Skorupski and Brian Bush (SPON: Brain Research Association). Dep. Neurobiology, Physiolology, University of Bristol, Bristol BS1 5LS, Bristol, UK.

In the crayfish thoracic ganglia subsets of the leg promoter and retromotor pools are excited in positive feedback reflexes resulting from leg movement. Group 1 promoter motoneurons are excited by the thoracocoxal chordotonal organ, which signals leg promotion, and group 1 retromotor motoneurons are excited by the thoracoabdominal muscle receptor organ, which signals leg remotion (Skorupski et al (1992), J. Neurophysiol. 67, 648-663). Other members of these motor pools lack these inputs and are excited by the opposite directions of movement, which correspond to negative feedback.

The amine octopamine, which is thought to be an inhibitory transmitter or neurotransmitter in in the crayfish swimmeret system (Mulloney et al (1987), J. Neurophysiol. 58, 584-597) also has inhibitory effects in thoracic ganglia. These effects are selective, however, and correlated with subdivisions of the motor pool on the basis of reflex input. At low concentrations (1-10x10-6M) octopamine disrupts or slows down any rhythmic activity and selectively depresses positive feedback reflexes. In the case of the promoter group 1s, excitatory TCCO input may reverse to become inhibitory. At high concentrations (50-100x10-6M) octopamine hyperpolarizes some group 1 motoneurons by 15 mV or more, with a reduction in input resistance of 60-70%. Group 2 motoneurons, at the same concentration, are depolarized by 1.5 mV, with little or no change in input resistance.

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592.15 GROWTH AND THE PASSIVE INTEGRATIVE PROPERTIES OF NEURONS. D.H. Edwards. Dept. of Biology, Georgia State University, Atlanta, GA 30302-4010

Study of the response properties of the lateral giant interneuron in large and small crayfish suggests that its PSPs are increasingly low-pass filtered as the cell grows. To test the effects of size increases on a neuron's passive integrative properties, the responses to current steps and maximum postsynaptic synaptic inputs were calculated for small and large (10X) equivalent cylinder models of neurons. Response rise-times and the attenuation of steady-state responses were significantly greater in the larger model, whereas the input resistance was 50-fold smaller. Increases in the injected current and maximum postsynaptic conductance restored the peak responses of the large model to the values of the small model, but did not affect the response attenuation or rise-times. Several-fold increases in the specific membrane resistance increased the large model's input resistance and reduced the steady-state attenuation, but also greatly slowed its responses. The reduced properties of the large model were restored to those of the small model when increases in specific membrane resistance were matched by similar decreases in the specific membrane capacitance.


Consummatory feeding behavior in Aplysia californica is generated in the buccal ganglia and regulated by the activity of specific central control neurons. According to this model, feeding behavior is initiated by sensory stimulation followed by a hyperpolarization. The hyperpolarization is generated in a region of the buccal commissure where the nerves to the buccal mass confluence. The hyperpolarization acts as a regenerative response, followed by a hyperpolarization. Depolarization of the soma produces a sustained, long-lasting response.


The B31/B32 cells in the buccal ganglia of Aplysia californica are previosuly shown to have many unusual electrophysiological features. The soma of these cells does not sustain conventional action potentials. Depolarization of the soma produces a sustained, long-lasting regenerative response, followed by a hyperpolarization. The hyperpolarization is generated in a region of the buccal commissure, close to neurons B20 and B4. These data suggest that the soma and axon of the B31/B32 cells are functionally compartmentalized. Information processing near the soma is accomplished by slow, regenerative potentials, while information processing in the axon and dendrites is via conventional action potentials. The role of these cells has yet to be established.

592.20 AFTERDISCHARGE ACTIVITY OF MOTORNEURONS INVOLVED IN FEEDING BEHAVIOR IN PTEROCOCC MOLLUSC. P.J. Morekiani* and R.A. Gattis, Dep of Zoology, Arizona State Univ, Tempe, AZ 85287 and Friday Harbor Laboratories, Friday Harbor, WA 98250.

The pterococ mollusc Clione limacina is a highly specialized carnivore which feeds on shelled pteropods and uses, for their capture, three pairs of oral appendages called buccal cones. Contact with the prey induces rapid eversion of buccal cones, and grasp the shell of the prey. A large group of electrically coupled, normally silent cells (motorneurons) has been described in the cerebral ganglia of Clione, whose activation induces oral anis odontophore cartilage and of the buccal mass. Feeding behavior is generated by a pattern generator comprised of three neuronal subunits (S1, S2 and S3). Each subunit acts independently, but can be linked temporally to either one or two of the other subunits to produce diverse motor patterns. The large intrinsic supralateral radial tensor (SLRT) muscle of the pharyngeal buccal mass plays a role in retraction, tensing, and hyper-retraction of the radula. The SLRT is innervated by at least 8 motoneurons. Two motoneurons driven by S3, and two motoneurons driven by S2 have been identified. Each motoneuron innervates a distinct region of the SLRT. Muscle contractions in different regions of the SLRT and at different times during the feeding cycle confer multifunctionality and allow flexibility during feeding behaviors. The SLRT neuromuscular system is therefore an excellent model to study the contributions of polynuclear innervation to complex behaviors.
INVERTEBRATE MOTOR FUNCTION

PATHWAYS ARE SUGGESTED.

Somatic muscle cells from the nematode Ascaris are electrically coupled and receive modulatory neural input from excitatory and inhibitory motoneurons. We have suggested that activity in contractile wave propagation is the pattern of electrical coupling observed between somatic muscle cells. Since several neurotransmitters have been implicated in gap junction channel modulation, it seemed of interest to test whether ACh or GABA had any effect on the gap junction channels of Ascaris muscle cells. Substances were bath applied while recording gap junction coupling coefficients in pairs of well coupled somatic muscle cells from anterior portions of the animal. Other compounds were also tested, including heptanol and octanol, which have been shown to decrease in close junctional channels in many preparations. GABA, 10 μM, hyperpolarized the cells, decreased the coupling coefficient and decreased the input resistance; ACh, 1, 10 and 100 μM, depolarized the cells and caused variable small changes in the coupling coefficient and input resistance; heptanol, 1 mM, induced a small decrease in the coupling coefficient and increased the input resistance, while octanol had negligible effect. The data suggest that gap junction channels from Ascaris somatic muscle are not modulated by the natural transmitters, and close in response to heptanol.

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593.5 GLUTAMATE- AND ASPARTATE-LIKE IMMUNOREACTIVITIES IN CHEMICALLY IDENTIFIED HYPOTHALAMIC NEURONS. B. Meister, A.P. Nicholas and T. Hökfelt. Dept. of Histology and Neurobiology, Karolinska Institute, SE-104 01 Stockholm, Sweden.

Recent evidence suggests that the amino acid glutamate (GLU) may be the dominant excitatory transmitter in neuroendocrine regulation. Both GLU and aspartate (ASP)-like immunoreactivities have been demonstrated in prehypothalamic axons. In the present study we have by means of indirect immunofluorescence histochemistry examined the distribution of GLU- and ASP-immunoreactive (IR) cell bodies within the hypothalamus. Of particular interest was to explore the presence of GLU- and ASP-like immunoreactivity (L-I) in chemically identified neurons of hypothalamic nuclei. GLU/ASP-L-I was demonstrated in many vasopressinergic (VP) and oxytocinergic neurons. Within the Arc, GLU/ASP-L-I was mainly found in the ventromedial aspect of the nucleus, however, single neurons were also distributed in the ventrolateral aspect. Double-labelled revealing that most of the GLU-L-I in the Y (NPOM) is colocalized with several peptides and TH in hypothalamic neurons. The results suggest that the excitatory amino acids GLU and ASP are colocalized with several peptides and TH in hypothalamic neurons.

593.7 SYNCHRONOUS NEURONAL ACTIVITY IN THE SURFACEAMICULAR NUCLEUS (SCN) INDEPENDENT OF CHEMICAL SYNAPTIC TRANSMISSION. Y. Bouskila and F. E. Dudek*. Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024

The SCN, which contains the mammalian biological clock, exhibits a circadian rhythm of firing rate. Since these data are derived from neuronal populations the approach that may reveal the synchronization in the SCN is in studies with defined mechanisms of synchronization. Using in vivo studies, we have examined possible mechanisms of synchronization in the SCN. L-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (KA) injection into SCN showed that the SCN is synchronized with similar bursts in the contralateral SCN (n=9). Furthermore, a mixture of NMDA, non-NMDA and AMPA receptor antagonists blocked the remaining synaptic potentials (n=4) and that the mixture of antagonists blocked the remaining spontaneous PSPs (n=6). These results indicate that spontaneous neuronal activity can occur in the SCN without active chemical synapses, suggesting that a different mechanism of communication exists; a similar mechanism(s) of neuronal synchronization may coordinate the cellular elements in the SCN responsible for circadian rhythm in mammals. Supported by AFSOF.

The suprachiasmatic nucleus (SCN) is thought to be the generator of circadian rhythms in mammals. Diurnal variations in the expression of several neuropeptides, including the one encoding vasopressin (VP), exist in the SCN, although the roles of these neuropeptides are not understood. VP receptors (of the V1a type that is present in hypothalamus and linked to phosphoinositide turnover) are also present in the SCN. Our cloning of the rat V1a receptor (VIaR; Nature 359, 523, 1992) enabled us to look for a diurnal rhythm in the expression of this receptor's gene.

Four groups of adult Sprague-Dawley rats (4 per group) were examined for 10 days (8 a.m. to 6:00-1800) prior to sacrifice at 0200, 1000, 1400, and 2200. 12a frozen sections through the SCN were processed for hybridization histochemistry for VIaR (Endocrinology 111, 1992) or VP (Mol. Brain Res. 1, 231, 1986) mRNA. We confirmed the diurnal rhythm for VP expression with highest levels at 1400. VIaR mRNA had just as steep a swing in levels, but peaked at 0200. (Statistical analyses were performed using ANOVA).

We are currently performing double-labeling experiments to see if the VIaR and VP transcripts are colocalized and are examining intact forebrain rats to see if the rhythm in VIaR expression is maintained.

ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS

PATTERN AND COLOR ENCODING NEURONS WITH OCULOMOTOR PROPERTIES IN THE MACAQUE PULVINAR. J.D. Port, E. Castillo and J. P. Micevych. Dept. of Neurosurgery, Univ. of California, Riverside, CA 92521 and Dept. Anatomy & Cell Biology, Univ. of California, Los Angeles, CA 90024

Recent evidence suggests that there is elevated direct intercellular communication in the suprachiasmatic nucleus (SON) during lactation. The major evidence for this is an increase in the incidence of dye-coupling in the SON of nursing mothers compared with virgin rats. Dye-coupling is thought to be mediated by direct intercellular channels of connexin protein, which in neurons has been shown to be connexin 32 (Cxn32). To test whether the increased coupling is related to elevated Cxn32 mRNA in the SON, virgin rats, mothers sacrificed before the start of lactation and mothers that had been lactating for 14 days were processed for Cxn32 in situ hybridization histochemistry with a 32P-labeled riboprobe (Micevych and Abelson, JCB 125:91-115, ’89). The labeling ratio was designated as density of silver grains over a structure + density of silver grains over the background. Cxn32 mRNA hybridization was observed over scattered cells throughout the SON in virgin rats (labeling ratio = 3.98 ≤ 1.20). No distinct dorso-ventral gradient was noted but most of the labeling was observed in the rostral to middle portions of the nucleus.

Mothers that were sacrificed after parturition but before lactation had the highest labeling ratio (10.61 ± 1.51) in the SON. Mothers that had been allowed to lactate for 14 days had a SON labeling ratio (6.62 ± 2.50). These results indicate that the Cxn32 mRNA increases prior to lactation and then is reduced during lactation and suckling. Thus, the neural and endocrine events at the time of parturition may be responsible for the elevated levels of Cxn32 mRNA which code for gap junction proteins that account for increased dye-coupling between SON neurons. (Supported by NS21220 and NS0140).


Bilateral lesions of either the medial prefrontal cortex (Adm) or mediiodorsal thalamic nucleus (MD) produce spatial context recognition deficits evidenced by a failure to respond to familiar but out-of-place stimuli (K.A. Stokes & P.J. Best, 1987, Neuroscience, 15:1067; J.M. Vargo & P.J. Best, 1988, Exp. Neurol., 112:199). Asymmetries in the behavior of rats with left vs right Adm lesions have been reported (J.M. Vargo & P.J. Best, 1989, Brain Res, 465:109). This study investigated the possibility that MD, the primary thalamic input to Adm, may be involved, with left MD lesions demonstrating more asymmetrical rotational behavior compared to right MD, bilateral MD, or control animals. Left MD animals also had a marked deficiency in visual 1080-1090) and would be expected to be the source of at least some of the asymmetry in Adm performance. The demonstration of greater contralateral spatial context recognition deficits in a unilateral version of the "out-of-place" paradigm. However, neither left nor right MD lesions produced semi-inattention as is seen following unilateral Adm lesions.
ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS

594.3 SEROTONINERGIC, NORADRENERGIC AND DOPAMINERGIC INNERVATION OF THE PRIMATE MEDIODORSAL THALAMIC NUCLEUS M.J. Schwartz* and L. Murias
Sect. of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Since many of the modulatory transmitter systems originating from the brainstem nuclei have similar actions on the activity of thalamocortical relay cells (McConnell, Pape, and Fitzpatrick, 1983), we examined the distributions of serotonin (5-HT), noradrenaline (NA) and dopamine (DA) immunoreactive fibers in the mediodorsal thalamic nucleus of the rhesus monkey. Our goal was to determine whether these systems have overlapping or distinct anatomical distributions. Immunoreactive axons of each of these systems exhibited a different and distinctive innervation was found for 5-HT labeled axons. These were present at all levels of the thalamus, predominantly in caudal portions of the nucleus. The most striking feature of the 5-HT fiber distribution was the irregular mosaic-like arrangement of areas containing higher and lower fiber densities. This mosaic-like organization was evident throughout the nucleus. In contrast to the innervation by 5-HT fibers, both the DA and NA innervations of the MD showed striking regional variations in their rostral-caudal and medial-lateral distributions. NA fibers were sparsely distributed throughout the nucleus, while NA fibers were more dense in the medial magnocellular subdivision. DA innervations were sparse in rostral portions of the MD. In caudal portions of the nucleus DA fibers were concentrated and numerous in ventral and lateral regions of the parvicellular division. Comparisons of 5-HT, DA and NA immunoreacted sections revealed that these two transmitter systems exhibited an interdigitating and complimentary pattern of innervation in the posterior thalamocortical relay systems originating in the brainstem have distinctive regional patterns of innervation in the MD which may contribute to the functional diversity of the subdivisions of this nucleus and its cortical targets. Supported by NS 28607-06 and MH 44866.

594.5 CHEMOARCHITECTONIC STUDY OF THE MEDIODORSAL THALAMIC NUCLEUS IN THE MACAQUE MONKEY C. Cavada*, T. Compagny, A. Hernández-González and P. Ponsuoso-Dugré
Dept. Morphology, Fac. Medicine, Univ. Madrid, 28040, Spain.

Histochemo- and immunohistochemical compartments were uncovered in the mediodorsal (MD) thalamic nucleus of macaques by analyzing the distributions of acetylcholinesterase (AChE) and cytochrome oxidase (CO) activities, and of serotonin (5-HT) and tyrosine hydroxylase (TH) immunoreactivities. Adjacent sections through the thalamus of adult Macaca nemestrina were stained to reveal Nissl substance and myelin, and processed for AChE and CO histochemistry, and for 5-HT and TH immunohistochemistry. Two AChE-poor, but CO-rich sectors were observed along most of the rostro-caudal extent of MD: the medial third, and the ventral rim adjacent to the centromedian nucleus. Within and between these sectors lie patches of moderate AChE staining. The lateral two-thirds of MD show prominent AChE activity, which is unevenly distributed: poor- and very rich-AChE areas are interdigitated with AChE-rich zones. CO staining in this region is less intense than in the medial third, and is distributed in dark and light zones. Many of the CO-rich zones appear to overlap with zones of AChE-moderate or rich staining. The rostral end of MD is predominantly AChE-poor, whereas the dorsal and caudal ends of the nucleus are predominantly AChE- and CO-rich. The 5-HT innervation of MD is prominent at the site of the thalamus. The distribution of 5-HT-IR fibers is heterogeneous, with denser aggregates usually coextensive with AChE-rich or moderate or rich zones. TH-in fibers in MD are relatively sparse, making it difficult to characterize heterogeneities in their distribution.

The present findings show that MD is a mosaic of biochemically diverse zones. This diversity is likely to have a bearing on the functional interactions of MD with the association areas of the frontal, parietal and temporal lobes. Supported by DGCYT PB88-0170.

594.7 ELECTROPHYSIOLOGICAL PROPERTIES OF CAT ASSOCIATION NEOCORTICAL CELLS. A Nulsen*, F. Amador and M. Stendal
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Intrinsic properties and synaptic responses of association cortical neurons were studied in vivo, by means of intracellular recordings in areas 5 & 7 of cats under urethane anesthesia. Cells were identified by ortho- and antidromic activation from extracellular stimulation of the MD thalamus and vicinity. Recordings were made with 5-30 MΩ "piggy-back" electrodes (single recording pipettes attached to seven-barreled iontophoretic assemblies). Twenty-eight percent of all cells showed an excitatory response to MD stimulation. Fifty-six cells that showed no excitatory responses were tested with the GABA_A antagonist bicuculline methiodide (BMM). BMM produced a short latency excitatory response in 37 (57%) of these cells.

Application of the AMPA antagonists CNQX or DNQX selectively decreased the excitatory responses. These data indicate that: (a) the thalamic projection to prefrontal cortex is excitatory, and (b) the excitatory projection is regulated by GABA. Supported by NIH Grant NS 16721.


Recent data have stressed the importance of certain calcium binding proteins (CBP) as markers for thalamocortical neurons, in particular Parvalbumin (PV) and 26-kd calbindin (CB). CBPS have been shown to be expressed in projection neurons in the thalamus and to distinguish separate classes according to their input-output connections. In the monkey thalamus, relatively few neurons fall into those of the reticular nucleus, show PV immunoreactivity (IR) (Jones &Hwyler, Eur. J. Neurosci., 2:227-240, 1990). We have observed that PV is more widely distributed in the GABA neurons of cat thalamus, which suggests certain interspecies variability in terms of neuronal specificity of CBPs. Little is known about the distribution of the CBPs in neurons of the intralaminar nuclei (IL). The aim of the present study was the immunocytochemical characterization of GABA-IR neurons of the cat anterior IL nuclei according to the CB and PV immunoreactive (IR) labeling. Immunohistochemistry double-labeling techniques have been used for the localization of the three antigens, GABA, PV and CB-IR. GABA neurons were distributed in a pericentral fashion within the IL nuclei. In superimposed adjacent sections processed for GABA, PV or CB, some degree of complementarity was observed, i.e., thin CB zones overlap with PV poor zones and vice versa. The distribution of GABA-IR is similar to that of PV-IR although the overlap was not complete. Double labeling experiments demonstrated that the majority of PV-IR neurons within layers I-V (up to 90%) were also GABA-IR, while only a small number of GABA-IR cells (10 to 20%) did not show PV-IR. Less than 30% of the total CB-IR neurons showed GABA-IR, while CB-IR was present in about 70% of the GABA-IR neurons. The present findings demonstrated that the majority of IL GABA cells contain at least one of the two CBPs PV or CB and possibly that CB and PV might coexist in the same GABA cells. Thus, GABA cells of the IL form a heterogeneous population in terms of their expression of CBPs, and present significant differences in comparison with monkey IL thalamic GABA neurons. This diversity may be related to the functional role of the IL nuclei in the two species.

GABAergic interneurons in the primate orbitofrontal cortex (OFC) is a component the paralimbic belt and consists of several distinct areas. Studies of the monkey OFC indicate that these areas are characterized by a complex array of intrinsic and extrinsic connections. To define the chemoarchitectonic organization of the human OFC, we have used antibodies to neuropeptide Y and to parvalbumin (SMI 32), and to the calcium-binding protein parvalbumin (PV). Immunohistochemical analysis of the bilateral cortical labeling patterns corresponding to the cytoarchitecture defined by Nissl preparations. SMI32-immunoreactive (ir) pyramidal neurons were demonstrated to be PV-ir in lamina rII and rIV. The presence of PV-ir although the overlap was not complete. Double labeling experiments demonstrated that the majority of PV-ir neurons within layers I-V (up to 90%) were also GABA-IR, while only a small number of GABA-IR cells (10 to 20%) did not show PV-IR. Less than 30% of the total CB-IR neurons showed GABA-IR, while CB-IR was present in about 70% of the GABA-IR neurons. The present findings demonstrated that the majority of IL GABA cells contain at least one of the two CBs PV or CB and possibly that CB and PV might coexist in the same GABA cells. Thus, GABA cells of the IL form a heterogeneous population in terms of their expression of CBs, and present significant differences in comparison with monkey IL thalamic GABA neurons. This diversity may be related to the functional role of the IL nuclei in the two species.

Physiological studies have demonstrated that the cingulate cortex contains a variety of subareas involved in the integration of sensorymotor functions. In order to characterize the complex anatomic organization of these regions, we have studied the chemocarchechemistry of the monkey as well as human cingulate cortex. Immunostaining with antibodies to nonphosphorylated neurofilament protein (SM132) and to the calcium-binding protein parvalbumin (PV) revealed striking regional differences and clear delineation of subareas in both species. For instance, layer III of areas 24b and 24c was practically devoid of SM132-immunoreactive (ir) pyramidal cells, whereas the posterior area 24c and 23c displayed an increasing density of these cells in both layers III and V. PV staining showed a bilaminar neuronal pattern anteriorly coinciding with layers III and V, and a more homogeneous labeling posteriorly as layer IV emerges in area 23. Interestingly, an area possibly corresponding to a cingulate motor region (area 24c) appears to be located at the transition zone between typical anterior and posterior subfields patterns and is characterized by the presence of numerous large SM132-ir pyramidal cells in layers III and V. In addition, in the human, large PV-ir pericellular baskets were observed in area V of area 24c. Thus, SM132 and PV exhibit regionally coordinate staining patterns that may represent anatomic equivalents of physiologically defined elements of the cingulate cortex.


We have recently shown that a subset of thalamic afferents terminate in the human cingulate cortex. The current study was undertaken to determine whether these afferents display any regional differences. Caudate and thalamic nuclei were labeled with biocytin following the intracortical injection of this tracer. Thirty one biocytin labeled somata were examined. Of these, 13 were found within layer I, 4 in layer II, 11 in layer III, and 3 in layer IV. The layer-specific distribution of biocytin labeled somata is consistent with the topography of the human somatosensory and motor cortex. In area 23b, the largest somatotopic representation was found in layer III, whereas layers II and IV contained few labeled neurons. In area 32, both the rostral and caudal portions contained well defined somatotopic representations, with the rostral portion displaying a more posterior representation. In area 24c, the posterior area displayed a smaller somatotopy than the anterior portion. These results are consistent with the idea that the human cingulate cortex contains several somatotopic representations.
ROSTRAL-CAUDAL TOPOGRAPHY OF SUBCORTICAL DIFFERENT PROJECTIONS OF THE MEDIAL AGNARULAR CORTEX IN THE RAT. J.V. Corwin, R.P. Mendel, S. Kins, and E. Leslie. Dept. of Psychology, Northern Illinois University, DeKalb, IL 60115, and Univ. of Wisconsin, Madison, WI 53706; and Dept. of Physiol. Sciences, Univ. of Florida, Gainesville, FL 32610.

Our previous research has indicated that unilateral destruction of the rostral or caudal medial agranular cortex (Agnm) produces dissociable behavioral components of the neglect syndrome. Given these behavioral differences resulting from rostral vs. caudal lesions it is of some interest to examine the anatomic connectivity of these components of the Agnm. In the present study we have focused on the rostral-caudal (r-c) topographic projections of the Agnm.

Assessment of the Agnm projections were produced by using WGA-HRP and serial reconstructions of the Agnm. A distinct r-c topography was noted for projections to caudate-putamen (c-p) and thalamus. Rostral injections produced terminal fields in the dorsal central core of the c-p rostral to the posterior commissure, injections in mid Agnm at the level of the septum labeled the dorsolateral rim of the c-p from the level of the genu to the fornix. Caudal Agnm injections produced sparse terminal fields in the dorsal c-p caudal to the genu. In thalamus, rostral Agnm project to the centriate lateralis (CL), ventral lateral (VL), gelatinous, and ventromedial (VM) nuclei; middle Agnm to the dorsal CL, and posterior (PO) nucleus; and caudal Agnm to PO and the ventral latersdorsal nucleus. Thus, the trend through Agnm is to demonstrate a rostro-ventral to caudo-dorsal pattern of thalamic labeling. The behavioral significance of these r-c differences in the afferents of Agnm remain to be determined.

594.17


The purpose of this study was to compare the activation patterns in the human brain of 1) discrimination versus matching as a psychophysical procedures and 2) intramodal (i.e. tactile) versus cross-modal (i.e tactile visual) matching. The regional cerebral blood flow was measured with 15O-butoranol and positron emission tomography in 9 normal subjects during 1) a control state, 2) a tactile discrimination task, 3) a tactile matching task and 4) a tactile visual matching task. The right hand was used for all tasks. The sensory stimuli were spherical ellipses (Roland & Mortensen, Brain Res. Rev. 12: 1-42, 1987). The contralateral postcentral gyrus and supplementary motor area, ipsilateral anterior lobe of cerebellum and anterior insula were activated in all four tasks. Discrimination exclusively activated the prefrontal cortex and contralateral head of the caudate nucleus. The tactile-visual matching was associated with the posterior inferior, parietal lobule bilaterally, the ipsilateral inferior temporal cortex and the ipsilateral pulvinar. The tactile-tactile matching task activated the ipsilateral posterior inferior temporal cortex and the contralateral precuneus. The results indicated that the comparison of two stimuli, in contrast to matching, require the participation of the prefrontal cortex. That the remote visual association areas are of importance for the conversion of tactile shape into visual representations and the representation of visual shape.

594.19

ENDOGENOUS POTENTIALS IN THE HUMAN ORBITALFRONTAL CORTEX EVOKED BY RARE AND REPEATED STIMULI. J. VoIkmann. Center for Neuromagnetism, Dept. of Physiology and Biophysics, New York University Medical Center, New York, N.Y, 10016, USA; +Dept. of Psychology, Open University, England.

Magnetic field responses were found at latency 100-140 ms, 150-250 ms, 260-290 ms. The dipole sources of their responses plotted on MRI suggested that they were located in the intraparietal sulcus.

594.20

ORIGIN AND CHARACTERISTICS OF COHERENT THALAMO-CORTICAL 40-HZ OSCILLATIONS IN THE HUMAN BRAIN. J. VoIkmann. Center for Neuromagnetism, Dept. of Physiology and Biophysics, New York University Medical Center, New York, N.Y, 10016, USA; +Dept. of Psychology, Open University, England.

A 14- and a 37-channel MEG system (BTI) were used, in order to determine the origin and the spatial-temporal dynamic properties of 40-Hz oscillations in the human brain. MEG data was recorded over an entire hemisphere (from 35-37 positions) before and during an auditory task. Magnetic 40-Hz responses were obtained from adult healthy subjects and from Alzheimer's patients. Magnetic Field Tomography (MFT) was located inside the brain (Ribary et al., Proc. Natl. Acad. Sci. 88, 11037- 11041, 1991). This technique comprises a mathematically weighted source space which allows the spatial localization of the source in the brain at different depth. MFT solutions were superimposed onto three-dimensional reconstructed brain images (MRI). Well-defined 40-Hz coherence was found between cortical-subcortical sites with a time shift that is consistent with thalamo-cortical conduction times. Significant data were also found in single recording epochs before and during auditory presentations indicating that the oscillatory activity in the 40-Hz range is continuously generated by the central nervous system, and is resetted by the auditory input. In addition, these single epochs suggest that these oscillations, like rostro-caudal progression of activity at thalamic and at cortical level. These activities were altered in Alzheimer's patients, where a similar activity pattern was present, but the cortical component was reduced.

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rhinal sulcus and entorhinal areas ventral to the rhinal sulcus, is considered to be a nodal point in the exchange of information between cortical and limbic regions. Behavioural results from primase studies suggest that the perirhinal cortex may play an important role in memory, as lesions of this area result in a severe memory impairment (Zola-Morgan et al. J. Neurosci., Vol 9(12) 1989). To date, there have been no studies investigating the function of the perirhinal cortex in the rat. In order to determine whether damage to this region produces a memory related deficit, bilaterally lesioned and control rats were tested in two paradigms. The initial procedure investigated the rats' response to novelty in order to test for deficits in spatial memory. It was found that rats with bilateral damage to the perirhinal cortex were not impaired at this task. In fact, latencies to find the platform were significantly lower than those of control animals. These results suggest that the perirhinal cortex, unlike other temporal lobe or limbic system structures, is not critically involved in spatial memory. Rather, it appears that this region may play some role in habituation or attentional processes.

Spatial Memory Deficits From Lesions of the Cholinergic Basal Forebrain Using Ibotenate, Quisqualate, and AMPA.

Spatial Memory Impairment following Adrenalectomy-Induced Dentate Gyrus Volume Reduction in Rats. C.D. Condon* and E.J. Roy. Neuroscience Program and Dept. of Psychology, Univ. of Illinois, Champaign, IL 61820.

Long-term adrenalectomy (ADX) has previously been shown to cause selective cell loss in the hippocampal dentate gyrus of the rat. Using stereological estimates, we quantified the volume of the dentate gyrus and investigated whether damage to the dentate gyrus impairs learning in two standard memory tasks (Morris Water maze and 8-arm radial maze). Rats were lesioned in both the medial septum and bilateral basal forebrain using stereotaxic techniques. Each lesioned group exhibited spatial learning and memory deficits in comparison to controls in the water maze. The behavioral deficits measured did not correlate with the degree of depletion of ChAT activity assessed in regions of cholinergic terminal fields.

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EFFECTS OF PARIETAL CORTEX AND HIPPOCAMPUS IN MEMORY FOR DISTANCE INFORMATION. J.M. Long, R.P. Kesner, Dept. of Psychology, Univ. of Utah, Salt Lake City, UT 84112. It has been postulated that rats form an internal representation of the environment as a spatial cognitive map (i.e., a distance, direction, spatial location) available to an animal in spatial tasks, it is not known which cues are represented in memory. In order to test for the contribution of the hippocampus and parietal cortex to memory for allocentric spatial cues, Long-Evans rats were trained in a go/no-go task which required the animal to remember the distance between two identical objects. After acquisition, rats were given either hippocampal or parietal cortex lesions, which resulted in impaired performance. In a different experiment rats were trained to discriminate the distance between two identical objects. Distance discrimination performance was impaired in rats with parietal cortex, but not hippocampal lesions. It appears that rats can represent allocentric distance information within their spatial cognitive maps. Furthermore, the hippocampus might be involved in temporary representation of allocentric distance information, whereas the parietal cortex might be the site of a more permanent representation of allocentric distance information.

PHTHALIC ACID LESIONS OF THE RAT NUCLEUS BASALIS MAGNOCELLULARIS (NBM) IMPAIR MEMORY IN A DOUBLE Y-MAZE. P.E. Mallet, R.J. Beninger, K. Jhamandas and R.J. Bogman, Depats. of Psychol. and Pharmacol. Toxicol., Queen’s University, Kingston, Canada, K7L 3N6. The differential effects of intra-NBM injections of excitotoxins on amygdalar choline acetyltransferase (ChAT) rather than cortical ChAT, may explain the differential mnemonic effects observed. The present study evaluated the mnemonic effects of intra-NBM infusions of phthalic acid (1,2-Benzendicarboxylic acid), an excitotoxin that strongly decreases amygdalar ChAT activity. Sprague-Dawley rats were trained to traverse a double Y-maze for food reinforcement, until performance exceeded 80% correct. Successful performance depended on accuracy in both a working and a reference memory component. Rats were then given either unilateral phthalic acid (300 nm, 0.5 μl) or sham (0.9% saline, 0.5 μl) lesions of the NBM, and were again tested on the maze. Biochemical analysis revealed that phthalic acid lesions produced a large decrease in amygdalar ChAT, but had little effect on cortical ChAT. Behavioral results showed an increase in working but not reference memory errors in the phthalic acid lesioned rats, and no effect in the sham operated controls. These results suggest that the cholinergic projections from the NBM to the basolateral amygdala play an important role in mnemonic functioning.

CUE FAMILIARITY REDUCES SPATIAL DISORIENTATION FOLLOWING HIPPOCAMPAL DAMAGE. J.E. Holden* and B. Tidswell. The University of Michigan, Ann Arbor, MI 48109. When the hippocampus (HPC) is damaged, place navigation is disrupted and spatial disorientation results. A single cue marking a hidden goal orientation in rats. However, when impaired were compared when to controls. We hypothesized that cue familiarity would enhance the ability of rats with HPC damage to locate the hidden platform in the Morris water test. After preoperative training with the cue (n = 21) or handling only (n = 17), rats were given electrolytic bilateral HPC (BHPC) lesions or sham surgery for controls. All rats were then tested for four days, six trials per day, with the cue marking the platform location. One way ANOVA showed that rats with BHPC lesions familiar with the cue (FB) were significantly more efficient at finding the platform than controls (p<0.05) and in directional heading error (X = 319, 4.40 vs 5792 ± 4.02 degrees, p<0.05) on Day 1. These differences occurred across the four test days. No significant differences were found between FB and controls for searching. A second experiment tested the effect of cue familiarity when a competing cue was present. Cue familiar animals were tested with a distractor in the testing environment postoperatively. No significant differences were found between FB (n=7) and FC (n=4) animals. We conclude that: 1) cue familiarity improves performance on a spatial memory task following HPC damage; and 2) the effect of cue familiarity remains in the presence of an environmental distractor.

THE EFFECTS OF LESIONS OF THE DORSAL HIPPOCAMPUS ON THE VENTRAL HIPPOCAMPUS ON PERFORMANCE OF A SPATIAL LOCALIZATION TASK. A.A. Chiba*, D.L. Johnson, and R.P. Kesner, Department of Psychology, University of Utah, Salt Lake City, Utah 84112. Each rat was trained on an eight-arm radial maze task examining memory for the temporal order of spatial location as a function of temporal distance. During the study phase of each trial, rats were allowed to visit each of eight arms once in an order that was randomly selected for that trial. During the test phase of each trial, rats were required to choose which of two arms occurred earlier in the arm sequence during the study phase. The arms presented in the test phase varied according to temporal distance (0, 2, 4, 6) or number of the arms in the running sequence of the study phase that occurred between the two test arms. Once the rats reached a criterion of 70% correct performance for the temporal distances of 2, 4, and 6 rats continued to display chance performance for the temporal distance of 0. Electrolytic lesions of both dorsal hippocampus, the ventral hippocampus, or the cortex immediately above the dorsal hippocampus (control lesions) were made. Following surgery, control lesioned rats continued to perform at chance for the temporal distance of 0, but performed at or above 75% correct for the temporal distances of 2, 4, and 6. The performance of rats with ventral hippocampal lesions did not significantly differ from that of control lesioned rats. The performance of rats with dorsal hippocampal lesions was at chance for both the temporal distances of 0 and 2, but did not significantly differ from that of control animals for the temporal distances of 4 and 6. These data suggest that memory for the temporal order of spatial location across all temporal distances is not dependent on the integrity of the ventral hippocampus but that memory for shorter temporal distances is dependent on the integrity of the dorsal hippocampus. However, previously reported data (Chiba & Kesner, 1989) revealed a performance deficit across all temporal distances (0, 2, 4, and 6) in rats with total hippocampal (dorsal and ventral) lesions. Thus, it appears that the effect of total hippocampal lesions in rats on memory for the temporal order of spatial location is not equivalent to the combined effects of independent dorsal and ventral hippocampal lesions in rats.

AMYGDALA LESIONS IMPAIR AND FORNIX LESIONS ENHANCE ACQUISITION OF INFORMATION ABOUT SPATIAL LOCATIONS: DEPENDENCE ON METHOD OF REINFORCEMENT PRESENTATION. B.J. McDonald* and N.M. White. Department of Psychology, McGill University, Montreal, Quebec, Canada. We have previously shown (McDonald and White, Neurosci. Abstr. 17, 54.1) that lesions of the amygdala, but not of the hippocampus or dorsal striatum, impair acquisition/retention of a conditioned cue preference using 2 arms of a 9-arm radial maze in which the arms were distinguished from each other by the presence or absence of a light at the entrance. In the present experiment we tested the effects of these lesions on a conditioned place preference using 2 randomly chosen arm locations (excluding adjacent locations) for each subject on a radial maze. The maze was physically rotated before each daily trial so the arms were distinguished solely by their spatial locations. Each pairing consisted of a session in which a rat was confined to one arm that contained food and a session in which the rat was confined to the other arm with no food, in a counterbalanced manner. On the test day, each rat was given free access to both arms in both locations for 20 min and the amount of time spent on each arm was recorded. Control rats showed a preference for the location associated with food after 3 pairings, but not after 1 or 2 pairings. Rats with bilateral damage to the lateral amygdala, but not to the hippocampus or dorsal striatum, were impaired on this task. Rats with damage to the fornix showed significant preferences for the food-associated location after 1, 2 or 3 pairings. These results suggest: 1) the method of reinforcer presentation may be a factor determining which neural substrate mediates acquisition of a memory; 2) when rewards are presented passively, acquisition of information about their relationship to neural stimuli may be mediated by a memory system that includes the amygdala. 3) when rewards are presented passively, hippocampal processing may interfere with acquisition even when the task involves learning about spatial locations.

A ROLE FOR THE LATERAL STRIATUM IN THE MEDIATION OF VISUOSpatial COGNITION. JCS Furtado* and MF Mazurek. McMaster University Medical Centre, Hamilton, Ontario, Canada. Recent studies have emphasized the importance of parallel pathways in the cortico-striato-pallido-thalamic circuit. In this model, the medial striatum is understood as being involved in "cognitive" processing while the lateral striatum is regarded as selectively mediating "motor" functions. We have studied the "cognitive" and "motor" abilities of rats with bilateral quinolinate-induced lesions of the medial (MED) or lateral (LAT) striatum. Compared with saline-injected controls, the experimental groups were significantly impaired in the place task version of the Morris Water Maze. No group differences were found when the platform was visible, suggesting that the observed impairment in the water maze reflects an impairment, on the part of the MED and LAT groups, to use visuospatial cues. By contrast, when the same animals were tested for motor behaviour (tongue extension and food manipulation) only the LAT group was impaired. This study confirms the selective role of the lateral striatum in the control of specialized motor behaviours, but suggests that both the medial and lateral striatum are involved in the mediation of visuospatial cognition.

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We hypothesized that unilateral cortex removal has two opposite effects pertaining to left-right response tasks: (1) provision of a pronounced neural asymmetry which facilitates acquisition, but also (2) induction of a unilateral response bias so strong that performance is impaired. We trained hemidecorated and sham-operated rats on two water-escape T-maze tasks, predicting that, in the hemidecorateds, mastery of the first task would break the induced response bias, thereby allowing the facilitation to appear on the second. The prediction was confirmed, but only for the right operators, who were indeed worse than the sham group before the first task, but when it was first, but better than the shams at either task when it was second. By contrast, left operators were worse than the shams at both tasks, irrespective of testing order. (Morriss-test performance was unaffected by either left or right hemidecoration.) The results confirm the hypothesis that induced neural asymmetry can facilitate left-right learning, and appear to reveal a left hemisphere specialization in rats, parallel to that in humans, for the ability to tell left from right.

595.15 THE ROLE OF POSTERIOR CINGULATE CORTEX (AREA 29) IN LEARNING AND MEMORY IN THE RAT. R. L. Sutherland*, L. H. Hoing, R. Kornewe and E. Evanson. Deps. of Psychology and Physiology, Univ. of New Mexico, Albuquerque, NM 87131-161. Area 29 is extensively connected with the hippocampal system (HPC), cortical association areas, and dorsal thalamus. Clinical studies and experimental work with animal models suggest that area 29 damage can produce amnestic symptoms. Our experiments were designed to explore the characteristics of the memory disorder and to clarify the anatomical basis for these effects. Prior work (L. Neary, 1980; Whishaw, 1973) with rats revealed that area 29 aspiration produces a long-lasting impairment of place learning using the Morris water task. We now demonstrate that: 1) destruction of area 29 impairs the intrinsic neurons of the cingulate gyrus, which is indistinguishable from nonspecific aspiration lesions. 2) Limiting the neural destruction to the subcallosal area, area 29c, produces similar, but less dramatic, impairments than total removal, 3) Using a combined lesion strategy including neurotoxic HPC, electrolytic anterior thalamic, and area 29 aspiration lesions, unilateral crossed lesions of area 29 + HPC or area 29 + anterior thalamus produce impairments comparable to magnitude to destruction of these structures bilaterally, 4) Increasing the interval between alcohol injection and area 29 damage does not affect the impairment, 5) Posterior parietal cortex, but not perithalamic, asperations produce anterior and retrograde impairments comparable to area 29 damage, and 6) Area 29 aspiration impairs the ability to resolve a nonspatial, negative patterned discrimination in a discrete trial barpressing task. Thus, area 29 cortex is important in certain forms of memory, acting in concert with HPC, thalamic, and posterior parietal circuitry.


Rats were trained on a matching non-matching test for spatial location, response and visual object information aimed at measuring working memory or delay-based memory for allocentric spatial, response (egocentric spatial) and sensory-perceptual (visual object) attributes. After training, rats received lesions of either the hippocampus, caudate nucleus or medial extrastriate visual cortex. After recovery from surgery the rats were retested. Results indicated that there was only a memory deficit for spatial location information even at the shortest delays for rats with hippocampal lesions, only a memory deficit for response information even at the shortest delays for rats with caudate nucleus lesions, and only a memory deficit for visual object information even at the shortest delays for rats with medial extrastriate visual cortex lesions. Thus, there appears to be a triple dissociation among the hippocampus, caudate nucleus and medial extrastriate visual cortex in mediating allocentric, response (egocentric spatial) and sensory-perceptual (visual object) attributes that support the neurobiology of an attribute model of memory.

595.18 DIFFERENCES IN SPATIAL AND VISUAL WORKING MEMORY PERFORMANCE OF RATS IN A PHD(±)-MAZE. W. J. Wilson*, R. Sergent, C. Leslie, P. Ouwschanski, S. Kellenberger, A. J. Birchall*, R. A. Bennett. Dep. of Psychological Sciences, Indiana University, Bloomington, IN 47405, USA.

Working memory in the rat has been studied extensively with tasks that involve spatial cues, often using the radial-arm maze or the T-maze. We compared working memory for spatial and visual information in two phd(±)-mazes, a model maze designed after the "automated T-maze" designed by Burg (1917), in which the rat is allowed to return to the Start Box for having chosen the correct arm. Rats were run in one of two visual presentation conditions in the phd(±)-maze, a model maze designed after the "automated T-maze" designed by Burg (1917), in which the rat is allowed to return to the Start Box for having chosen the correct arm. Rats were run in one of two visible presentation conditions in the phd(±)-maze. In the Spinal task, rats were reinforced for alternation between the left and right arm. All rats in this task reached the criterion of 15 or more correct trials on two successive daily 20-trial sessions within 16 sessions. In the Visual task, rats were reinforced for alternation between the lighted and dark arms. None of the 6 rats in this task reached the criterion of 15 or more correct trials on two successive daily 20-trial sessions; when performed at high level, the trial did not alter the rats. Thus, they were able to perform a task that required working memory of visual information, but were unable to use visual working memory, despite being able to discriminate based on the stimuli. This suggests that the rats differ in their capacity to encode spatial (prospective, kinesthetic) and visual information in working memory, or the contralateral present spatial cues interfered with learning of the visual task. 

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Knife cuts to the perforant path (PP) disrupt acquisition of spatial (place, locale) learning in the Morris water maze (Skelton & McNamara, 1992 Hippocampus, 2, 78). The present study examined retention and recovery of both spatial navigation and place learning.

Rats were trained to locate a submerged platform, then given bilateral knife cuts of the PP, either in the angular bundle (1.7 mm ant. to EBZ) or 2 mm further anterior (to denervate only the dorsal hippocampus). Starting 48 hrs after surgery, rats were tested with 4 submerged platform trials, 1 probe trial and 1 visible platform trial daily for 14 days, then weekly for 6 weeks. Rats with cuts at either level of PP were initially impaired on submerged platform and probe trials, but not on visible platform trials. Performance on probe trials reached control levels after about 10 test days, and continued to improve over the 6 weekly tests. A residual deficit was revealed when the platform was then moved to a new location: rats with cuts at either level took 3-4 days to reach the level of probe trial performance reached by controls in 1 day. These results suggest that 1) damage to the PP impairs the dorsal hippocampus may be as debilitating as total PP transaction 2) the PP is required for accurate navigation and place recall, 3) substantial recovery or retraining of spatial navigation occurs within 10 days, and 4) recovery of place learning remains incomplete for up to 2 months. (Supported by research grant from NSERC.)


The present study was designed to assess in rats the effects of ischemia-induced damage on the performance of two 8-arm radial maze tasks: one (spatial win-shift) sensitive to lesions of the hippocampus, and the other (cued win-shift) sensitive to lesions of the caudate. Male Wistar rats received either sham surgery or PP transient forebrain ischemia induced by a combination of bilateral carotid occlusion and hemorrhagic hypotension. Following an 8 week recovery period the rats were tested on one of the two tasks. Each trial of the win-shift task consisted of two phases. In phase 1, 4 target arms were blocked and rats were allowed to enter and retrieve food from the 4 unblocked arms. In phase 2, all arms were unblocked and the previously blocked target arms were baited. Rats were allowed to enter the maze until all 4 target arms had been visited. Each rat received daily trials with a delay of 4s between phase 1 and phase 2, until they reached the criterion of 4 target-arm entries in the first 5 arm entries during phase 2, for 3 consecutive trials. Rats then received 15 trials with a 5-min delay, and 6 trials with a 2-h delay. A random set of 4 target arms was used on each trial. Ischemic rats were significantly impaired at both delays, but not at the 4s or 5-min delays, indicating that ischemia affects performance of this spatial win-shift task only at long delays.

In the cued win-shift task, all 8 arms were accessible, with light cues at the distal ends of 4 target arms. On each daily trial, rats were allowed to explore the maze until they had entered and retrieved food from each lit arm twice (arms were rebaited after the first entry). After a rat retrieved food from a lit arm for the second time, the light was turned off. The random set of 4 target arms was used on each trial. Ischemic rats were not significantly impaired on this cued win-shift task.

These findings of ischemia-induced impairments on a win-shift task at long delays, but not on a cued win-shift task suggest that this model of ischemia causes functional damage to the hippocampus, but not to the striatum.

596.3 AGED C57 MICE DECLINE IN SPATIAL LEARNING PERFORMANCE AND ASSOCIATED HIPPOCAMPAL PROTEIN KINASE C ACTIVITY. J.M. Wehner*, D.E. Fordyce and J.M. Weinber. Institute for Behavioral Genetics, University of Colorado at Boulder. Boulder, CO. 80309-0447

C57Bl/6J and DBA/2J mice were tested on the Morris water maze task for 6 days followed by 12 days of testing on the place-learning-set task (8 trials/day with each task). Mice were tested at 3, 14, and 25 months of age. A decline in learning performance was observed in mice 15 and 25 months of age compared to mice 3 months of age. Mice 3 months of age showed no significant effect of age on either performance. These aged mice also demonstrated a significant reduction in membrane-bound hippocampal protein kinase C activity (p<0.05) with no significant change in cytosolic or loosely-bound protein kinase C activity. The effects of aging in C57 mice on spatial learning performance and hippocampal protein kinase C activity were found in both the Berg and Nos strains. These data, therefore, indicate that the age-induced decline in spatial learning performance in C57 mice may be associated with an age-induced alteration in hippocampal protein kinase C activity.

Supported by NSF BNS-882 and NIH HD-07289 postdoctoral training grant.


The effects of physical activity on spatial learning performance and associated hippocampal functioning were examined in C57Bl/6 and DBA2J (B6) mice. Previously, we observed a marked enhancement in spatial learning performance and associated alterations in hippocampal cholinergic function of rats exposed to a period of activity (Wehner et al., 1991. Beh. Brain Res. 46:125-133). Because of genetic analyses afforded by using inbred strains of mice if was of interest to extend our previous work to a more widespread study of the effects of physical activity on the hippocampus. A protocol consisting of moderate activity (0.4 mph) treadmill running 5 days/week and 60 min/day. Mice were then tested on the Morris water task for 6 days followed by the place learning-set task for 12 days (6 trials/day with each task). Hippocampal protein kinase C activity was measured via cysosolic, loosely-bound and membrane-bound homogenate fractions. Mice subjected to the physical activity protocol were compared to age-matched sedentary controls from the same set of littermates. Physical activity enhanced performance on both learning-set tasks (p<0.02) in B6 mice, which characteristically perform poorly in comparison to C57 mice, were enhanced to perform similarly to C57 mice. These alterations in performance were accompanied by alterations in membrane-bound protein kinase C activity (p<0.05). The data from this study, therefore, indicate that the protein kinase C second messenger system, as well as cholinergic function, in the hippocampus may be involved in the physical activity-induced enhancement of spatial learning performance.

Supported by NSF BNS-8820076 and NIH HD-07289 postdoctoral training grant.

596.5 MOUSE STRAIN DIFFERENCES IN LEARNING SET SPATIAL NAVIGATION PERFORMANCE. E.F. Paton, Dept. of Psychology, Red Deer College, Red Deer, Alberta, Canada T4N 5H5.

Swiss Webster (SW); DBA; and Deer mice (DM) were tested for acquisition and retention of learning set place task in the Morris water maze. The learning set consisted of daily placing the hidden platform sequentially at 1 of 4 separate locations in the pool. All animals were flown for 63 days in this version of the water task. SW mice were unable to reliably find the platform. The time taken by DBA and DM in escaping the pool declined rapidly, reaching asymptote within 21 days, with DM showing the ability, throughout the study, to reach the platform significantly faster than either SW or DBA. Analyses of swim path selection used by the 3 strains clearly indicated that DM were the most systematic in the selecting and sequencing, from a variety of potential strategies, the appropriate methods necessary for the most efficient solution of the problem. The present results suggest, that in light of the strain differences observed, further investigation of strain differences in the neuroanatomical structures believed to be related to the solving of spatial problems, might be a fruitful area of investigation.


Sex difference in spatial ability have been proposed to be related to mating systems, space use, and reproductive condition. Deer mice, Peromyscus maniculatus, are polygamous rodents with reproductive males displaying greater range sizes than females. The present study considered the effects of reproductive status on both spatial learning by males and females of two different populations of deer mice. The performance of the two populations of adult breeding and non-breeding deer mice was examined in a Morris water maze. Latency of the animals to reach the hidden platform was measured over 6 blocks of 4 trials. All groups of mice were able to learn the spatial task, with the mice derived from a population originally present in an arid interior region acquiring the task more slowly than mice that were descendants of a population inhabiting a small island. In both populations, breeding males showed significantly faster acquisition and better retention of the spatial task than did breeding females. In contrast, non-breeding mice of both populations displayed no significant sex differences in their spatial learning. This indicates that reproductive and hormonal condition have significant effects on the display of sex differences in spatial learning.
ACQUISITION, LEARNING-SET, AND LONG-TERM RETENTION DEFICITS OF SPATIAL INFORMATIONS IN AGED MICE. C. Lehman, I. Koezig, and R. Jaffard. Laboratoire de Neurosciences Comportementales et Cognitives, URA 339 and Laboratoire de Neurobiologie des Ceregles, Univ. Bordeaux 1 et II, Avenue des Facultés, 33405 Talence, France.

Ability in learning and memory during aging in mice has been investigated using a food-choice discrimination task in a 8-arm radial maze. Each discrimination consisted of presenting two adjacent arms (pairs) with only one always the same, baited across trials. On the first phase, animals were concurrently trained on two (Acquisition 1) until they reached a criterion of 13 correct responses out of 16 successive trials. On the second phase (Acquisition 2), animals learned to discriminate the other pairs. To acquire the task, the animals had the possibility to use either extra-maze (place) or extra-maze cues (black and white visual cues constantly placed on each arm across the trials).

Results showed that, as compared to young mice (4-5 months), aged animals (24-25 months) exhibited slower acquisition on the first two pairs (Acquisition 1), ii) weaker positive transfer from Acquisition 1 to Acquisition 2, iii) a long-term retention deficit (over 20 and 40 days) of previously acquired informations (Acquisition 1 and 2).

An additional experiment was conducted in order to verify the contribution of intra-maze cues to discrimination performance in young and aged mice. Results showed that young mice use preferentially extra-maze cues, whereas aged mice use both intra- and extra-maze cues. Supported by CNRS, URA 339, and Fondation "France Alzheimer".

LITTER SEX-RATIOS AFFECTS ADULT PERFORMANCE IN A SPATIAL TASK. I.A. M. Gales*, K.-P. Ossenekopp, & M. Kavaliers. Neuroscience Prog., Univ. of Western Ontario, CANADA.

Previous research in this laboratory has shown that breeding adult meadow voles Microtus pennsylvanicus acquire a spatial task in a sexually-dimorphic manner, favoring males, and that no sex differences are shown in performance in immature voles. The present study addressed the question of whether there would be sex differences in performance on a spatial task in breeding adult voles which had previously not demonstrated a sex difference when tested as juveniles. Sixteen litters of adults were re-tested in a hidden platform Morris water-maze six weeks after being initially tested at either day 10, 15, 20, or 25 after birth. Juvenile voles tested at days 20 or 25 acquired the task faster as juveniles compared to the adult group. Sixteen litters of adults were re-tested in a hidden platform Morris water-maze six weeks after being initially tested at either day 10, 15, 20, or 25 after birth. Juvenile voles tested at days 20 or 25 acquired the task faster as juveniles compared to the adult group.

Litter sex-ratios affected adult performance in a spatial task.

MEMORY DEFECTS AND ASSOCIATED DECREASES OF CEREBRAL 2-DG LABELLING INDUCED BY CHRONIC ALCOHOL CONSUMPTION IN MICE: REVERSAL BY METHYL β-CARBOXYLASE-3-CARB-XYLATE ADMINISTRATION. B. Bonetti, D.J. Berachois, C. Destreza and R. Jaffard. Laboratoire de Neurosciences Comportementales et Cognitives, URA CNRS 339, Univ. Bordeaux 1, Av. Facultés, 33405 Talence, France.

We previously showed that long-term (17 months) chronic alcohol consumption by mice produced significant reduction in cerebral energy metabolism as measured by 2-DG labelling in the diencephalon, in particular the mammillary body (IBM; Soc. Neurosci., 1991, Abs. 17, 481). Here, we investigated the effects of two shorter durations (6 and 12 months) of alcohol consumption by Balb/c mice on 2-DG uptake patterns observed following a memory test in a T-maze. 2-DG was injected into the jugular vein immediately before a 35 min period of testing for sequential alternation in a T-maze and the subjects were sacrificed for autoradiographic analysis using the relative 2-DG method. Although no significant effects on either 2-DG labelling or memory performance were observed after 6 months of alcohol consumption in comparison to controls, mice of the 12 month group showed significantly increased susceptibility to interference in the alternation task. This mnemonic deficit was associated with a concomitant decrease in testing-induced 2-DG labelling intensity of the MB. Administration of β-CCM (0.5 mg Kg−1 s.c.) to subjects of the 12 month alcohol group reversed the memory impairment of these mice as compared to saline-injected controls. Parallel studies of the effect of β-CCM on 2-DG labelling patterns are being conducted in an attempt to establish an eventual correlation between memory performance in the alternation task and MB metabolic activity.
A.G. Gittis* and M. McHaddon, Psychology Department, Westminster College, New Wilmington, PA 16152.

Two choice spatial alternation procedures have been variously called "win-shift" tasks or delayed non-match to sample. Variants of this procedure are used to assess functional and behavioral mechanisms under the assumption that working memory is needed to remember a prior response and that shifting is an aspect of working memory. For example, on a delayed matching to sample (DMS) task, working memory is needed to remember a prior response and that shifting is an aspect of working memory. For example, on a delayed matching to sample (DMS) task, subjects were assigned to groups in which, for an additional 100 trials, reward for the incorrect arm remained the same, whereas reward for the correct arm was increased. After acquisition, subjects were assigned to groups in which, for an additional 100 trials, reward for shifting increased to 21. Results indicated that present results in light of observations that alternation requires little prodding when it appears counterintuitively. A resolution is offered by interpreting alternation through the 'dead reckoning' model proposed by Gallistel (Organization of Learning, 1990).

The Johns Hopkins University, Baltimore, MD 21218.

The present experiment introduces a variable interval (VI) probe trial to assess spatial memory in the water maze. A popular construct in recent theories of spatial performance is that rats use visual distal cues to create a "cognitive map" of the maze surroundings. Previous research has indicated that rats will either use a distal-cue (spatial) strategy or a pattern-recognition (non-spatial) strategy to solve a working memory task in the radial arm maze. In the present experiment we used a novel testing paradigm to test the hypothesis that rats which use the spatial strategy require the presence of distal cues. Each male rat was first trained on a working memory task on an eight-arm radial maze with all visual distal cues present. Following the attainment of an 85% accuracy criterion for the probe phase, the rats were then tested on the maze with all the visual distal cues blocked by a black curtain. Results indicated that of the 7 rats that used a spatial strategy during the initial training phase, 4 of these rats continued to use an apparent spatial strategy during the retest phase although no visual cues were present. Our results indicate that spatial strategy need not be derived exclusively from distal cues. Additionally, one of the rats which initially used a response strategy during the training phase continued to use the strategy during the retest phase.

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596.16


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FORTH VENTRICAL BOMBESIN INJECTIONS SUPPRESS SUCCROSE INTAKE IN DECEREBRATE RATS. F.W. Flynn*
Dept. of Psychology and Neuroscience Program, Univ. of Wyoming, Laramie, WY.

Systemic injections of bombesin (BN) suppress sucrose intake in decerebrate rats. Also, 4th ventricle injections of BN in intact rats inhibit feeding. These studies clearly demonstrate that BN-produced afferent signals are both detected and integrated by central brainstem (CBS) and forebrain circuits to control intake. To evaluate whether the CBS, in isolation of the forebrain, contains the requisite neural systems to mediate the effects of 4th ventricle BN injection on ingestive behavior, intraoral sucrose (0.1 M) intake was measured in control and decerebrate rats (n=11/group) following 4th ventricle injections of 1, 5, 10, 20, and 50 ng BN. Fourth ventricle BN injections produced the same reliable dose-dependent reductions of sucrose intake in both groups. This result, taken with a previous report that systemic injections of BN suppress ingestive behavior in decerebrate rats, provides compelling evidence that local CBS neural circuitry mediates the sensory-motor integrative aspects that underlie bombesin's effects on ingestive behavior. (supported by NIH NS24879)
597.7 NALOXONE AND FLUOXETINE BLOCK EATING IN A PYY-MODEL OF BULIMIA NERVOSA. Z. H. Hasha and D. E. Moss*. Laboratory of Psychoneurochemistry, University of Texas at El Paso, Texas 79968.

Peptide YY (PYY) has been implicated in the neurobiology of bulimia (Kaye et al., 1990; Morley et al., 1987). Naloxone (NAL) and fluoxetine (FLU) have been proposed as anti-bulimic agents (Ezra, 1989; Mitchell, 1988). Little is known about the effect of NAL and FLU on PYY-induced eating.

Female rats with cannulae in the fourth ventricle were pretreated with NAL (100ng/rat i.c.v), FLU (10mg/kg i.p., Vehicle (VEH) 5-10mg/kg p.o., clomipramine (CLO) 3-30mg/kg i.c.v., CLO 5-10mg/kg p.o., or vehicle (VEH). Each rat received 13 lavage tests in total. Twenty minutes after intracerebroventricular injection of 2ug PYY, each rat had access to Food, every hour for a total of 15 g PYY. Cumulative food intake for PYY alone was 8.02g. VEH intake was 3.22g. Both central and peripheral injections of NAL blocked PYY-induced eating (3.21g). FLU also suppressed PYY-induced eating (3.29g). Central injection of FLU, however, did not affect PYY-induced eating (6.36g). Both central and peripheral CLO also had no effect on PYY-induced eating (6.35g).

Our results with NAL suggest opioids mediate PYY-induced eating. The results with FLU and CLO indicated that a serotonergic mechanism may be involved but at a site distant from the fourth ventricle and via FLU-sensitive 5-HT receptors. Further research is being directed at other neurochemical locations.

[Fluoxetine gift from Eli Lilly. Supported by MRDP 47167.]


Non-peptide CCK receptor antagonists abolish suppression of real and sham feeding by exogenous CCK and some intestinal nutrients. They also increase real feeding when injected in the absence of exogenous CCK. For these reasons, we have studied the effects of a potent, heptapeptide, CCK receptor antagonist. Since this antagonist is protected by blood-neural barriers, therefore, we have examined the effects of exogenous CCK on the licking behavior of rats drinking 4% sucrose following the injection of various clinically used anorectic drugs and CCK-8. Although acute injections of CCK-8 suppress food intakes in rats, when the compound is administered on a daily basis, progressively higher doses are required to affect intakes as animals become tolerant to its anorectic actions. We examined in more detail the development of this tolerance.

Four groups of male rats were placed on a restricted feeding schedule where they were allowed access to a liquid diet for 45 min in the AM and for 30 min in the PM until intakes had stabilized. During the subsequent training period, rats were injected (ip) daily with either vehicle (V) or CCK-8 (10nmol/kg) 10 min prior to the drinking session and again 2h after it. Administration of CCK-8 prior to feeding suppressed (p < 0.05) 60 min intakes about 25% on the first injection day but had no effect by Day 3. Ten min prior to feeding on Day 4 (test day), one group of rats was administered V, while the other 3 were given CCK-8. Analysis of 60min intakes indicated that CCK-8 on the test day suppressed feeding in rats that had received it after the feeding period during training, but not in those that had not received it. The effects of CCK-8 were most similar to those of FLU and FEN; more detailed analyses will be presented.

Conclusions. Under our conditions, FLU did not reverse the inhibition of food intake produced by CCK-8. We found that the development of tolerance was slower in 4h deprived rats than in rats maintained on the feeding schedule described above. Our results suggest that learning mechanisms and the animal's motivational state may be involved in the development of tolerance to the anorectic effects of CCK-8.

597.11 EFFECTS OF CCK-4 AND VARIOUS ANORECTIC DRUGS ON THE LICKING BEHAVIOR OF RATS. K. E. Asin*, J. D. Davis2 and L. Bednarz1, Neuroscience Res. Div., Pharmaceutical Discovery, Abbott Labs, D-47U Bldg-APIO, Abbott Park, IL 60064 and 2Univ. IL-Chicago, Dept. Psychol., Chicago, IL 60680.

Although acute injections of CCK-8 suppress food intakes in rats, when the compound is administered on a daily basis, progressively higher doses are required to affect intakes as animals become tolerant to its anorectic actions. We examined in more detail the development of this tolerance.

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The present experiments further explored the effects of GRF on macronutrient intake, since opiate blockade in this site blocks expression of GRF-induced feeding. This feeding effect is into the SCN/MPOA during the middle of the light cycle stimulates protein (P) intake up to 4 hours post-injection; the effects on C intake were inconsistent. The next study examined the ability of selective deprivation to alter the feeding effects of either SCN/MPOA GRF (1 pmol) or 2 mg/kg MOR. Rats were tested in ad lib groups). GRF increased P intake in ad lib and P deprivation conditions. C deprivation further enhanced P intake following C deprivation. A final study examined involvement of GRF in expression of circadian patterns of macronutrient selection. Animals were injected in counterbalanced order with 0, 1, 10, 17 nmol/kg at 1 h (50%, P < 0.01) and 2 h (35%, P < 0.01), by 2 nmol/kg at 1 h (42%, P < 0.05), 2 h (49%, P < 0.01), and 4 h (20%, P < 0.05); and by 2 nmol/kg at 2 h (24%, P < 0.001) and 4 h (17%, P < 0.01). The differences in amino acid sequence between rat and human IAPP (6 of 37 amino acids) may be sufficient to impair binding of human IAPP to the receptor involved in the feeding response.

Chronic treatment with amphetamine enhances the prophyagic effect of the kappa-opioid agonist U-50,488H (USO50). Since, cross-sensitization between stress and amphetamine has been reported, we studied the effect of chronic stress on the feeding response to USO50. An automated apparatus was used for the continuous monitoring of feeding, drinking, and locomotor activity. Eight male Wistar rats underwent ten daily (6 h after the onset of the light phase) stress sessions (20 min of restraint) whereas 8 control animals were only briefly handled. All rats had food and water ad libium. There was a slight increase in food intake in stressed animals in the 30 min following stress. After the last stress session the rats were left undisturbed for 2 consecutive days in a counterbalanced order, each animal being injected i.p. with saline on one day and with 3 mg/kg of USO50 on the other day. USO50 increased feeding and reduced drinking in the following two hours; more rats ate (100% vs. 62.5% after saline) and ate a larger first meal. The prophyagic effect of USO50 was greater in stressed rats. Food intake in the first hour was 2.8±0.37 g in stress USO50 vs. 1.7±0.41 g in group Control-USO50. The size of the first meal (intermeal interval ≥ 30 min) was 3.19±0.46 g in group Stress-USO50 vs. 1.79±0.35 g in group Control-USO50. The antidepressive effects of USO50 was unchanged. These results demonstrate that chronic exposure to a relatively mild and non-anorectic stress can sensitize the rat to the prophyagic effect of the highly selective kappa-opioid agonist U-50,488H.
597.19
THE EFFECTS OF CONTINUOUS MORPHINE INFUSIONS ON DIET SELECTION AND FOOD INTAKE A.J. Gosnell and D.D. Krain., Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109.

The administration of morphine can cause a short-term increase in food intake, and repeated administration of morphine has been shown to cause progressively larger increases in intake and/or the relative intake of dietary fat. In this experiment, we measured the effects of continuous morphine infusions on diet choice and total intake. Male rats were given ad lib access to two diets: a high-carbohydrate diet (CHO; 80% carb., 20% protein) and a high-fat diet (FAT; 80% fat, 20% protein). Diet intakes were measured daily for 21 days. Via the implantation of osmotic minipumps, one group (n = 15) received continuous infusions of morphine sulfate (approx. 2.8 mg/kg/hr) for days 1-7, and of saline for days 8-21. A second group (n = 11) was infused with saline for days 1-7 and with morphine for days 8-21. A third group (n = 12) received sham surgery but no minipumps. Regardless of whether morphine was infused when the diets were introduced, after 7 days of adaptation to the diets, morphine caused a significant decrease in CHO intake for the first 5 days of the 7-day infusion period (compared to the sham group). Intake of the FAT diet was generally elevated at the beginning of the drug infusion period and depressed toward the end of this period. Total caloric intake was significantly decreased on final 6 days of morphine infusions. Percentage of total caloric intake consumed from the FAT diet was significantly decreased for only the first 2 days of the infusion periods. Termination of morphine infusions caused a decrease in the intake of both diets, with no change in relative diet preference. Intakes generally returned to pre-drug levels within 1-3 days. These effects (a sustained decrease in total intake with only an initial increase in fat preference) differ from those reported after repeated intermittent administration of morphine in short-term trials. Supported by NIDA Grants DA05471 and DA05627.

597.20

Saccarin intake in rats is decreased following naltrexone (NTX) and increased following mu and delta agonists. Our laboratory recently evaluated the central roles of general (NTX), mu (beta-funaltrexamine, B-FNA), kappa (nor-binaltorphimine, Nor-BNI), and delta agonists (naltrindole, NTI) on opioid antagonists upon intake of sucrose which possesses palatable and nutritive qualities. Sucrose intake was reduced by NTX, B-FNA and Nor-BNI, implicating mu and kappa receptors. The present study evaluated over 1 h central antagonist effects upon intake of saccharin which possesses palatable qualities without postigestive consequences. NTX (20-50 μg) significantly reduced saccharin intake (25-50 min, 60-67%) confirming opioid involvement. In contrast, neither B-FNA nor Nor-BNI affected saccharin intake, suggesting that mu and kappa effects upon intake altered postigestive factors. Saccharin intake was significantly reduced by DALCE (5-15 min, 51-60%) and NTI (5-60 min, 65-80%), implicating delta receptor mediation. Since sucrose and saccharin differ in their postigestive consequences, any opioid involvement in palatability should consider post-ingestive factors in assessing receptor mediation. (Supported by DA04194).

598.1
SEROTONIN POTENTIATES ETHANOL-INDUCED EXCITATION OF VTA NEURONS IN VITRO. M.S. Brodie and S.A. Sather. Dept. Physiology and Biophysics, The University of Chicago, Chicago, IL 60637.

Dopamine neurons of the ventral tegmental area (VTA) are important components of brain pathways which mediate drug-induced reward. Ethanol (EtOH) increases the firing rate of VTA neurons in vivo (Gessa, et al., 1985), and in vitro (Brodie, et al., 1990). The EtOH-induced increase in firing rate of VTA neurons may underlie the rewarding effects of ethanol. It has been shown that serotonin receptors blockers reduce alcohol intake in human alcoholics and animal studies. We have begun studies to assess the effects of serotonin (5-HT) on ethanol-induced excitation of VTA neurons.

Cerebral brain slices containing the VTA were prepared from young adult rats (100 to 200 gm), and the slice was superfused in a recording chamber maintained at 35-36°C.Twenty-one neurons from 17 rats were studied. All drugs were administered in the superfusate. Concentrations of ethanol (40 - 160 mM) were tested in the presence and absence of 5-HT (1 - 50 μM). All neurons studied were excited by EtOH, and had electrophysiological characteristics typical of putative dopamine-containing neurones. In most cases, serotonin alone had a small, transient excitatory effect on these neurones; this excitation subsided within 20 minutes. In 15 of 21 neurones (71%), the excitatory effect of ethanol was increased in the presence of 5-HT by more than 10%. Increases in EtOH-induced excitation of 100% over pre-5-HT controls were seen typically, while the largest increase in EtOH excitation observed was 821%. These studies suggest that serotonergic agents may be useful in altering the potency of EtOH in reward areas of the brain and may provide an opportunity for the development of pharmacotherapeutic agents useful in the treatment of alcoholism. Supported by F.I.S. grant #AA-A05846-09.

598.2
EVALUATION OF M-CHLOROPHENYLPIPERAZINE (mCPP) EFFECTS ON ETHANOL (E) INTAKE AND BEHAVIOUR IN WISTAR RATS. Q.M. Tomkins, Y. Buszek and E.M. Seller*. Departments of Pharmacology, Medicine and Psychiatry, University of Toronto and Clinical Research and Treatment Institute, Addiction Research Foundation, Toronto, Ontario M5S 2S1, Canada.

The 5-HT1 agonist, mCPP, has been reported to produce subjective effects similar to those following alcohol intake and increase desire for alcohol in chronic alcoholics. The aim of the present study was to assess the effects of mCPP on E intake and the behavioural profile exhibited by the rats prior to and during an E limited access procedure. Following an acquisition phase, rats were placed in individual drinking cages at 16:30 h each day and 15 mins later were given 40 mins access to 12% E solution and water. The rats' behaviour profile exhibited by the rats prior to and during an E limited access procedure. Water intake was unaffected. On vehicle-treated days, rats exhibited a consistent behavioural profile. Following 1 mg/kg mCPP, prior to E access, rats competed more and exhibited fewer tube directed behaviours (p < 0.05). Rats with no preference between 0.07 ± 0.01 g/kg also exhibited a similar shift in their behavioural profile. Thus, mCPP caused a marked attenuation in E intake in E-prefering rats. At the lower doses investigated this reduction was selective and not due to behavioural disruption. The effect on preparatory behaviour at 1 mg/kg may reflect a reduced anticipation for E. Alternatively, the anxiogenic properties of mCPP may underline this profile as is supported by our observations in the low drinking rats.

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598.3 ETHANOL PRODUCES NALOXONE-BLOCKABLE ENHANCEMENT OF EXTRACELLULAR DOPAMINE IN NUCLEUS ACCUMBENS OF LEWIS RATS. E.L. Gardner* and J. Chen, Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, New York, NY 10467. Activation of the mesolimbic dopaminergic system appears to constitute the essential reinforcement produced by abusable substances (Wise, Pharmacol. Biochem. Behav. 13[suppl.1]:213-223, 1980), and brain dopamine (DA) systems project to the nucleus accumbens (Acc) are hypothesized to constitute a crucial substrate for brain reward (Wise & Bozarth, Science 283:253-256, 1999), essential reinforcement produced by abusable substances (Wise, 1982). However, the role of ethanol on these brain systems has been less clear than for other abusable drugs. We now report that acute ethanol challenge (0.25-5.0 g/kg, i.p.) induces a dose-dependent increase in basal extracellular levels of DA and its main metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in Acc of freely moving Lewis rats as measured by in vivo brain microdialysis. Naloxone at 0.1 mg/kg i.p. produced 50% blockade of this effect, and naloxone at 0.5 mg/kg i.p. produced 100% blockade. These data confirm previous microdialysis findings of ethanol-induced activation of forebrain DA systems (Di Chiara & Imperato, Proc. Nat. Acad. Sci. U.S.A. 85:5724-5728, 1988), but additionally implicate endogenous opioid peptide mechanisms in ethanol's activation of brain reward substrates. Thus, the present data may help to explain previous findings that naloxone blocks ethanol's enhancing effects on electrical brain-stimulation reward (Lorenz & Sainati, Life Sci. 23:1359-1364, 1978). (Supported by NIH grant RR 05397, NIDA grant DA 03622, and a research grant from the Aaron Diamond Foundation).


The role of the mesolimbic dopamine system in drug reinforcement processes has received wide attention over the last several years. However, the role this system may play in ethanol reinforcement remains to be elucidated. In this study, rats were trained to lever press for oral ethanol (10% v/v) reinforcement using a sucrose-substitution procedure. They were neither food nor water restricted during the experiment. When stable responding was achieved, bilateral cannula guides were implanted 1 mm above the ventral tegmental area (VTA). Following recovery, quinpirole was infused at doses of 0.001, 0.01, 0.1 and 1.0 μg/brain injected into the VTA were tested at weekly intervals. At doses of 0.1 and 1.0, responding was significantly decreased compared to both saline and sham control sessions. The decrease in responding was primarily a result of an early termination of the normal response pattern. This effect was identical to that observed in prior research on the response pattern following microinjection of the D2 DA antagonist raclopride into the n. accumbens. The results suggest that a blockage of DA transmission in the mesolimbic dopamine system at either the VTA or the n. accumbens reduces ethanol reinforced behavior. This supports the hypothesis that ethanol reinforcement involves this mesolimbic DA pathway as implicated for other drugs of abuse.

598.5 SENSITIZATION TO THE DOPAMINE RELEASE-ENHANCING EFFECTS OF ETHANOL DEMONSTRATED IN MALE LONG-EVANS RATS. D. Benjamin*, E.R. Grant, R.R. Goldstein, and L.A. Pohorecky, Center of Alcohol Studies, Rutgers University, New Brunswick, NJ 08903. In contrast to Wistar and Sprague-Dawley rats, dopamine (DA) release in the n. accumbens (NA) of male Long-Evans rats does not increase in response to an initial exposure to ethanol (ET). The present study characterized the ET-induced repeated exposures to ET on the release of DA, serotonin (5-HT), and their metabolites using intracerebral microdialysis coupled with HPLC-EC. ET-naive male Long-Evans rats were implanted with guide cannulae over the NA and the ventral tegmental area (VTA). One week after surgery, microdialysis probes with active membrane lengths of 2.5 mm were lowered into the NA and 2 mm in the VTA. The probes were perfused with Ringer's solution at a rate of 1 μl/min. On the first day of dialysis, baseline amounts of neurochemicals were determined over 3-5 samples at 20 min intervals, after which saline was injected IP. Following one or more post-saline samples, ET (1.0 g/kg, IP) was injected and 4 samples were collected, then a second 1 g/kg injection was given. This procedure was then repeated 24, 48, or 72 h later. We have found that, in Long-Evans rats, DA release in the NA does not increase in response to ET subsequent exposure to 1 g/kg caused 383±11% (p < 0.05, n=6) increments in DA release in the NA 24 h later. Substantial increments were found at the later timepoints. These results demonstrate increases in the mesolimbic DA system to ET with repeated exposure, and suggest that increases in DA release in the NA might underlie sensitization to the reinforcing/rewarding effects of ET in Long-Evans rats (see Goldstein et al., this volume). Further characterization of the mesolimbic DA sensitization to ET is in progress. (Supported by Smithers Prevention and NIAAA grants AA05306 and AA08499).


The importance of central monoamines, with the emphasis on the dopaminergic neurons, in the control of voluntary ethanol consumption was examined by studying the effect of ethanol on the extracellular levels of monoamines in the nucleus accumbens of the alcohol preferring AA (Alko Alcohol) and alcohol avoiding ANA (Alko Nonalcoholic) rats with in vivo microdialysis. Samples were collected from freely moving animals every 15 minutes, and concentrations of the monoamines and their metabolites were determined in the dialysate with smallbore HPLC. Ethanol (0.5, 1, or 2 g/kg, IP) significantly increased the extracellular levels of DOPAC and HVA in a dose dependent manner suggesting stimulation of dopamine release by ethanol. A similar trend was found with dopamine itself although the results from the present material did not reach significance. The extracellular levels of 5-HT, 5-HIAA or noradrenaline were not affected.


Intracellular recordings in hippocampal brain slices were used to evaluate the effects of ethanol on 5-HT mediated membrane hyperpolarization and spike afterhyperpolarizations (AHPs) in CA1 pyramidal neurons. Acute ethanol (30 mM) treatment in vitro nor in vivo ethanol dependence affected 5HT-mediated (1-100μM) hyperpolarization. Acute ethanol (30mM) increased AHPs during the 40 min superfusion making its apparent biphasic effect on 5-HT-mediated hyperpolarization of AHPs difficult to interpret. Most striking was the complete block of ethanol-induced enhancement of the AHP by 5-HT (100μM). In vivo ethanol dependence did not alter 5HT inhibition of the AHP. These results suggest that ethanol may interact with pre-sumed 5-HT receptors thought to mediate AHP block in the hippocampus, while 5-HTM receptors appear relatively resistant to ethanol. Supported in part by AA06222 and R01DA, AA0101 to GDF.

598.8 BEHAVIORAL EVIDENCE FOR DOWN-REGULATION OF SHT3 RECEPTORS PRODUCED BY CHRONIC ETHANOL. C.W. Walls, S.M. Rezagah*, and H. Lat. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX, 76107.

Ethanol treatment increases the efficacy of ligand gated ion channels; thus it may result in down regulation of such receptors. In the SHT receptor family, only SHT3 receptors are located on ligand gated ion channels. MLD2222 (MLD, a SHT3 receptor antagonist, 10 μg/kg, i.h) produced no effect on the time spent in the open arms of an elevated plus maze (EPM, Lal et al., Alcohol 8:467-471, 1991) in naive rats, but significantly reduced the total arm entries. In these rats, MLD (10 μg/kg) given 1 h prior to an injection of ETOH (1.5 g/kg, ip) resulted in a reduction of ETOH-induced hyperactivity without changing the rate of recovery (additive with ETOH tolerance). Chronic ETOH treatment (7 d in liquid diet, 4.5%), open arm and total arm activity was reduced in animals trained to discriminate between MLD and ETOH (3 g/kg). Animals given MLD (5,10, or 20 mg/kg) showed a further reduction in open arm and total arm activity, demonstrating exacerbation of these symptoms of ETOH withdrawal. MLD (10mg/kg) given to naive animals trained to discriminate pentyleneetetrazol (PTZ) from saline (Lal et al., JPR 247:508-518, 1988) resulted in a reduction of the threshold dose for PTZ discrimination. ETOH withdrawal also reduced the threshold dose for PTZ discrimination and this effect was additive with that of MLD. It is hypothesized that ETOH treatment results in down regulation of SHT3 receptors as part of the development of tolerance and/or dependence. Supported by NIAAA Grant AA06890.
98.9 EXTRACELLULAR CONCENTRATIONS OF DopAc, HVA and SHIAA IN NUCLEUS ACCUMBENS ARE CORRELATED WITH MEASURES OF ETHANOL-SEEKING BEHAVIOR IN RATS. B.A. Blanchard, S. Wang, S. Stinus and S.D. Glick, Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208.

The mesolimbic dopamine (DA) system has been proposed as a neuronal substrate for reinforcing effects of drugs of abuse such as stimulants, opiates and ethanol (EtOH). Rats with high EtOH preference, as a group, have been reported to have lower levels of DA and STR in nucleus accumbens (NAC) relative to non-prefering rats. We examined the role of individual differences in DA, DopAC, HVA and SHIAA in NAC and striatum (STR) in individual differences in EtOH consumption. Levels of these compounds were assessed in freely moving adult male and female Long-Evans rats by in vivo microdialysis and HPLC. Rats were then trained to bar press for oral EtOH reinforcement. Intake of and preference for 10% (v/v) EtOH were significantly correlated with several neurochemical measures in NAC: DopAC, HVA and SHIAA, but not DA, were inversely correlated to measures of EtOH-seeking behavior. There were no significant correlations of STR metabolites with EtOH intake. These findings indicate that individual differences in EtOH intake are related to differences in neurotransmitter regional levels in the mesolimbic system, providing further support for its role in the reinforcing properties of EtOH.

Supported by NIAAA grant A089599 to SDG.

98.10 EFFECTS OF CHRONIC ETHANOL IN NMDA-INDUCED RELEASE OF \( \text{H} \)NORADRENALINE IN THE RAT HIPPOCAMPUS. J. Labarca, B. Sepulveda, M. Seguel, W. Rosenthal, Dept of Medicine and Biological Sciences, Catholic University, Santiago, Chile.

Recent findings support the notion that ethanol-induced behavior is mediated, at least in part, by the NMDA/ligand complex. For this reason, we decided to study the effects of chronic ethanol administration on the function of NMDA receptors using microdialysis techniques. In addition, we studied the release of hippocampal slices as a functional model.

In CA1-CA3, ethanol reduced the excitatory projections of 10, 20, 50 and 100 mM, evoked the release of aspartate (ASP), the highest concentrations being the most potent. The omission of Ca²⁺ ions from the superfusion media completely abolished the ethanol-induced effect. The effects of ethanol on the release of GLU was much weaker, and only observed at higher concentrations of ethanol. At 20 mM of GLU ethanol enhanced \( \text{E} \)voked release of ASP but not that of GLU.

Twenty four hours after a chronic liquid ethanol diet for 3 months, NMDA-evoked release of \( \text{H} \)Noradrenaline was marginally augmented in the dentate gyrus (DG) but not in CA1-CA3. After 30 days of withdrawal, however, the NMDA-evoked release of \( \text{H} \)Noradrenaline in the DG was markedly inhibited, whereas in CA1-CA3 a trend of this phenomenon was observed, without reaching statistical significance. These results suggest that chronic ethanol consumption produces changes in NMDA/ligand complex that may be related, at least in part, to the pharmacological effects of ethanol. (Supported by PONDECy No 0739/91).

99.1 ACUTE AND CHRONIC COCAINE ADMINISTRATION: DOSE RESPONSE EFFECTS ON ZIF268, C-FOS, PREPROENKEPHALIN, AND PREPRODYNORPHIN mRNA IN RAT BRAIN. J. Daunais, W. Bohler, and J.F. McGinty, Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, N.C. 27858.

Wistar rats were administered saline or cocaine (NICDA) i.p. at 10, 20, or 30 mg/kg once per day for 10 days. After the last injection, the rats were anesthetized and decapitated. Brains were removed and frozen until later use. Sections were cut on a cryostat, fixed, dehydrated, and hybridized with a 35mer oligonucleotide probe to preprodynorphin (PPE), or a 40mer oligo probe to c-fos or zif268 at 37°C for 12 hr. After stringent washing, the slides were dried and exposed to Kodak X-OMAT film for 2 wk (PPD) or 1 wk (PPE, c-fos, zif).

ZGG8 mRNA levels decreased in a dose-dependent fashion after acute and chronic cocaine in layers IV and V of frontal and parietal cortex, layer II of piriform cortex, olfactory tubercle, dorsal striatum, nucleus accumbens, olfactory tubercle, and tenia tecta. Acute cocaine induced a far more robust signal than chronic cocaine in these areas. Minimal induction of ZGG8 hybridization signal was present in the nucleus accumbens, particularly in the core of cocaine-treated animals after acute or chronic cocaine treatment.

In the acutely treated rats, c-fos mRNA increased dose-dependently in piriform cortex, olfactory tubercle, and dorsal striatum, whereas signal was lacking in the chronic cocaine groups, except in the piriform cortex, indicating a downregulation of c-fos.

PPE and PFD signal hybridization were robust in the dorsal and ventral tecta of all groups. Digital analysis is currently under way to determine if there are any appreciable differences in signal between the cocaine-treated animals and their respective controls after acute or chronic administration. These data suggest that widespread changes occurring at the molecular level after acute cocaine may differ from those following chronic cocaine. Supported by DA03862.

99.2 AP-1 AND CRE-BINDING ACTIVITY ARE REGULATED IN THE RAT LOCUS COERULEUS AND NUCLEUS ACCUMBENS FOLLOWING CHRONIC MORPHINE OR COCAINE. B. Hope*, H. Nye, and E.J. Nestler, Lab. of Molecular Psychiatry, Dept. of Pharmacology and Cell Biology, Yale School of Medicine, New Haven, CT 06508.

We have studied changes associated with transcription factors, which control gene expression, in two model systems of drug addiction in the rat: chronic morphine in the locus coeruleus (LC) and chronic cocaine in the nucleus accumbens (NAc). Correlating with increases in c-fos and c-jun in the LC during morphine withdrawal (Brain Res., 522:256), AP-1 binding in gel shift assays has now also been shown to decrease. A decrease in CREB phosphorylation with acute morphine, and an increase following morphine withdrawal has been shown recently in the LC (J. Neurochem., 58:1168). We now report that there is no change in CRE-binding during acute morphine, and that a persistent increase in CRE binding following chronic cocain (Hope et al., PNAS, 1992), there is also an increase in CRE-binding, which increases even further following 1 week of withdrawal. There are also striking regional differences in CRE-binding patterns in gel shift assays, further indicating regional heterogeneity of transcription factors in the brain. The results indicate that levels of transcription factors are altered in the brain following chronic drug treatment, and that such alterations may underlie functional changes associated with addiction.

99.3 ACTIVATION OF TRANSSCRIPTION FACTOR GENES IN STRIATUM BY COCAINE IS MEDIATED BY BLOCKADE OF BOTH 5-HT AND DA UPTAKE. B. Blanchard, B. Blanchard, and J. Blanchard, Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Cocaine elicits robust increases in the expression of several transcription factors in striatum. As this response is abolished by 5-HT receptor blockade, it has been assumed that blockade of DA uptake mediates the striatal activation of transcription factors. However, we have found that the selective 5-HT uptake inhibitors, fluoxetine (5-10 mg/kg) and citalopram (2 mg/kg) which do not potentiate c-fos or zif268 by themselves, markedly potentiate the ability of amphetamine, a blocker of DA uptake, to activate c-fos or zif268 in striatum. These findings suggest that cocaine's blockade of 5-HT uptake contributes to its activation of these genes. To assess the role of the 5-HT system, rats were treated with p-chlorophenylalanine to lesion the 5-HT system selectively. This treatment markedly reduces activation of zif268 and c-fos by cocaine in the striatum. In conclusion, lesioning of the NE system with DSP-4 does not result. These results indicate that, along with the DA system, the 5-HT system plays a key role in mediating cocaine's activation of transcription factor genes in the striatum.

The involvement of both these systems may help explain several anomalous features of the pharmacology of this response.

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598.5

THE USE OF SUBTRACTION HYBRIDIZATION TO DETECT COCAINE UP-REGULATED MESSENGER RNAs IN RAT NUCLEUS ACCUMBENS. J.R. Walker*, E.J. Nestler, and K.A. Sevarino, Division of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale Univ.

School of Medicine, New Haven, CT 06510.

Cocaine is one of the most reinforcing substances known. Further, chronic use results in sensitization to the drug, a phenomenon which may be related to its powerful addictive effects. Behavioral, electrophysiological, and biochemical evidence indicates that the nucleus accumbens (NAc) is part of the neural pathway mediating the reinforcing and sensitizing properties of cocaine. To identify protein alterations in the NAc that underlie cocaine's effects, we have developed a subtraction hybridization procedure to detect cocaine-regulated mRNAs. Subtraction enrichment was performed by hybridizing cDNA synthesized from cocaine-treated NAc mRNA with mRNA from untreated rat NAc. The subtracted cDNA was used as a probe to screen a rat NAc cDNA library, and sequences representing mRNAs up-regulated by chronic cocaine were isolated. The mRNAs detected to date fall into three groups: several novel proteins, mitochondrial-encoded proteins, and artificially isolated ribosomal RNAs. We are currently characterizing the cocaine-regulated proteins, both known and novel, for their pharmacological specificity to cocaine, the time course of their regulation, and the anatomical pattern of their regulation. We are also improving the subtraction hybridization protocol to eliminate the artifactual detection of ribosomal RNAs.

598.7

CHARGE ISOFORMS OF DOPAMINE TRANSPORTERS. R.A. Vaughan, M.T. McCoy and M.J. Kuhar*, NIDA Addiction Research Center, P.O. Box 1580, Baltimore, MD 21224.

Dopamine transporters from rat striatum and nucleus accumbens membranes were photoaffinity labeled with either 125I-DEEP, (a GBR analog) or 125I-RTI-82 (a cocaine analog) (Brain Res. 1992, 596:173). Radiolabel-pure preparations were subjected to isoelectric focusing in one dimensional urea-polyacrylamide slab gels in order to investigate sample heterogeneity. Following electrophoresis, radioactivity corresponding to dopamine transporters was distributed into several sharp bands of approximate pI 5.6-6.6. Very similar patterns were obtained using tissue from either region and either photoaffinity probe. Since the predicted isoelectric point of the protein based on cDNA sequence is 7.05, the multiple acidic forms observed may be a consequence of heterogenous post-translational modifications. Enzymatic deglycosylation and dephosphorylation of dopamine transporter samples are being used to test this hypothesis.

598.9

COCAIN RECEPTORS SOLUBILIZED FROM RHESUS STRIATUM ARE HETEROGENEOUS ON THE BASIS OF CHARGE. L.M. Gracz*, L. Markham*, R.B. Reith, and R.B. Rothman*. NIDA Addiction Research Center, P.O. Box 5180, Baltimore, MD 21224.

Cocaine receptors solubilized from monkey striatal membranes were studied for their heterogeneity of binding sites. [3H]CFT binding was fully inhibited by cocaine congeners, monoamine uptake inhibitors, and dopamine in each peak. Additionally, the peaks exhibited different high- and low-affinity binding profiles for cocaine, GBR 12935, and dopamine. However, the individual DEAE peaks could not be resolved by size-exclusion gel chromatography. The results suggest that cocaine binding site heterogeneity may be a function of the charge state of the dopamine transporter. Supported by USPHS grants DA00499, DA06303, MH14175, and RR00168.

598.10

CATHODIC AND ANIONIC REQUIREMENTS FOR THE BINDING OF [3H]CFT TO THE DOPAMINE UPTAKE CARRIER. M.L. Rahn* and L L Coffey. Dept. of Basic Sci., University of Illinois College of Medicine, Peoria, IL 61656.

[3H]CFT binds to the dopamine transporter with a higher affinity than its parent molecule [3H]coclaine. In the present study with freshly prepared Sprague-Dawley striatal P2 membranes, [3H]CFT binding occurred to a single site. When Na+ was the only cation present at 1-200 mM and phosphate the co-variant anion in incubations at 4°C in 55.2 mM sodium phosphate buffer, pH 7.4, with a protease inhibitor cocktail. In order to obtain data suitable for quantitative curve fitting, it was necessary to periodically repurify the [3H]ligands by HPLC. Under these conditions, we observed greater than 90% specific binding. The method of binding surface analysis was used to characterize the interaction of GBR12935, BTPC, mazindol, and CFT with binding sites labeled by the [3H]ligands. Fitting of the data to one and two site binding models, using MLAB-PC, demonstrated that for both [3H]ligands, the two site model fit the data far better than did the one site model. Structure-activity studies suggested that while each radioligand labeled a site common to both, each also labeled a distinct site. These results support the hypothesis of multiple binding sites/states associated with the DA transporter. Identification of selective agents for these sites may be valuable tools for further studies of the DA transporter.

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*p < .01, **p < .05 compared to paired value at 0 HgCl₂, one-tailed paired t-test.

EFFECTS OF INJECTION OF KINASE INHIBITORS INTO THE A10 DOPAMINE REGION ON COCAINE-INDUCED MOTOR ACTIVITY. J.D. Staknis

Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Acute peripheral injection of cocaine stimulates motor activity in parallel with an increase of extracellular dopamine in the nucleus accumbens, as measured by in vivo microdialysis. The enhanced behavioral and neurochemical responses which occur with repeated, intermittent treatment are termed sensitization. We have shown that blocking a PKC- and protein kinase A (PKA) -dependent pathway in protein kinase C (PKPC) activity has been associated with sensitization. Before examining the role of PKC in sensitization, we were interested in the effects of different kinase inhibitors on the acute motor-stimulant response to cocaine. Cannulae were bilaterally implanted above the ventral tegmental area (A10 region), a region proposed to be critically involved in the sensitized behavioral response to cocaine, for intracranial injections. One week after surgery animals received intra-A10 injections of 1 μM H7 kinase inhibitor 5 min after receiving peripheral injections of saline or cocaine (15 mg/kg). Animals received each of the 4 possible treatment combinations with a minimum 72 hr inter-trial interval. The kinase inhibitors used in these studies included polymyxin B (PMB), a calmodulin kinase and PKC inhibitor, H7 hydrochloride, a PKC and protein kinase A (PKA) inhibitor and H8 hydrochloride, a PKA and cGMP-dependent protein kinase inhibitor. Preliminary data demonstrated that H7 dose-dependently inhibits, while PMB does not alter cocaine-stimulated motor activity. The most effective dose of H7 (30 nmol/ side) did not significantly alter baseline motor activity. These data suggest that PKA may play a role in the acute motor-stimulant response to cocaine and will be verified with intra-A10 injections of H8.

In vivo microdialysis studies are currently being conducted to determine whether the H7-induced inhibition of cocaine-stimulated motor activity, is associated with an inhibition of cocaine-induced dopamine release in the nucleus accumbens.

EFFECTS OF CHOLERA TOXIN INFUSION INTO THE NUCLEUS ACCUMBENS ON LOCOMOTOR BEHAVIOR. S.T. Cunningham and A.E. Keiley

Dept. of Psychology, Northeastern University, Boston, MA 02115.

Intracellular signal transduction mechanisms are currently the focus of much research. Although manipulation of second messenger systems is widespread in cell biology, there are very few experiments examining the consequences of such manipulation on behavior. In three separate experiments, we investigated the effects of microinjection of cholinergic toxin (CTX, a bacterial toxin that stimulates production of cyclic AMP) into the nucleus accumbens (N. Acc.) on locomotor activity in rats (N=30). For Experiment I, three groups of rats received either saline or CTX (50 or 500 ng) into the N. Acc. Locomotor activity (horizontal and vertical activity) was measured for 4 h following a single CTX infusion and subsequently for 4 h on consecutive days. No acute effects on motor activity were observed. However, the 500-ng dose of CTX induced long-lasting hyperactivity that was apparent 24 h later and that lasted 4 days. A smaller but significant hyperactivity occurred on days 4 and 5 following the 50-ng dose. Site-specificity of this behavioral phenomenon was investigated in Experiment II by infusion of CTX (250 ng/μl) into either the N. Acc. or the posterior dorsal striatum (PDS). CTX treatment of the PDS had no behavioral effects, while the low dose CTX infused into the N. Acc. was replicated. In Experiment III, the effect of intra-accumbens pretreatment with saline or CTX (10 ng/μl) on amphetamine (0.5 mg/kg)-induced motor activity was investigated. This low dose of CTX did not increase baseline motor activity one day later; however, CTX did induce a sensitized locomotor response to amphetamine. These data suggest that CTX induces long-lasting upregulation of the cyclic AMP system which is reflected by enhanced motor responses normally mediated by the N. Acc. Further, the results may have important implications for mechanisms underlying drug-induced sensitization.
Localization of Aging-Related Changes in α2-Adrenergic Receptors to Specific Brain Regions in Fischer 344 Rats

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Pharmacology, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262.

Aging-related regulation of central α2-adrenergic receptors was examined in specific brain regions of 8- and 27-month-old Fischer 344 rats. For these studies, saturation isotherms were generated with the partial α2-adrenergic receptor agonist p-[3H]-iodoclonidine (1-2 picomoles) and qualitatively autoradiographic analysis. Assays were carried out in 10 mM MgCl2 and 1 mM EGTA buffer (pH 7.4) containing 10 mM MgCl2 and 1 mM EGTA for 90 min at 21°C. In the three brain regions investigated—cerebral cortex, hypothalamus, and locus coeruleus—1-2 picomole bound to a single class of noninteracting sites. The affinity of the receptor for 1-2 picomole did not differ among the three brain regions or between the two age-groups; the Kd values ranged from 0.4-1.5 nM. The rank order of the density of α2-sites was hypothalamus > locus coeruleus > cerebral cortex. The density of receptors in the hypothalamus of the aged rats was reduced by approximately 60% (adult: 1350 ± 162 fmol/mg; aged: 561 ± 155). In contrast, no significant aging-related reductions were observed in either locus coeruleus (adult: 482 ± 144; aged: 350 ± 59) or cerebral cortex (adult: 246 ± 17; aged: 212 ± 21). These results demonstrate that there is a marked loss of α2-adrenergic receptors in the hypothalamus of aged Fischer 344 rats, which is not observed in either locus coeruleus or cerebral cortex. The receptor down-regulation in the hypothalamus may contribute to altered behaviors in aged animals. Further, in locus coeruleus and cerebral cortex, functional changes, rather than changes in receptor affinity or density, would be expected to mediate aging-related changes which involve α2-adrenergic receptors. (Supported by USPHS AG04418)

Behavioral Analysis of Chronic Nimodipine Treatment in Aged Rats

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Nimodipine is a calcium channel antagonist reported to have beneficial effects on treatment of ischemic damage as well as the potential for retarding aspects of brain and behavioral aging when provided chronically to rats (A. Scabini et al., FASEB J. 3:1799, 1989). We treated aged male F-344 rats (22-24 mo) with nimodipine in subcutaneous pellets in the following doses: 0 (controls), 20 mg (low-dose) or 40 mg (high-dose) replenished after 6 wk. After 3 mo of treatment, surviving rats and a group of young controls (6 mo) were tested in a behavioral battery involving exploratory activity in an open field and in a narrow wheel cage as well as remaining on an inclined screen, suspended from a wire, and balanced on a rotorod. Rats were also pretrained for one-way active avoidance in a straight runway before the open field test. During 20 trials rats were required to negotiate each of 5 maze segments within 10 sec to avoid footshock (0.8 mA). According to analysis of variance (ANOVA), nimodipine treatment produced no significant effects (p>0.05) on body weight, intake, or survival of aged rats. ANOVA of behavioral results indicated significant (p<0.05) age-related decline in performance of all tasks except in open field behavior. Nimodipine treatment had no significant effects on performance of aged rats except in maze learning. Rats on the high-dose regimen performed significantly (p<0.05) better than aged controls in the maze. The results indicate that chronic nimodipine treatment of aged rats had no toxic effects and might be beneficial for preventing age-related decline in learning performance.

Nimodipine Decreases Calcium Action Potentials in Aging and Young Rabbit CA1 Neurons

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The dihydropyridine calcium antagonist, nimodipine, facilitates learning in aging rabbits (Deyo et al., 1989) and increases spontaneous firing of CA1 neurons in vivo (Thompson et al., 1990). We previously reported that 100 nM nimodipine reduced the post-burst afterhyperpolarization (AHP) of aging but not young CA1 neurons and that aging neurons had larger AHPs (Moyer et al., 1991). Since the AHP is primarily a Ca2+-activated K+ current, we sought to understand the effects of nimodipine on calcium action potentials (APs) in aging and young CA1 neurons.

Intercellular recordings were made from 18 CA1 neurons (11 young, 7 aging) using current-clamp. 2M CsCl electrodes were used to block voltage-dependent K+ currents and 4 mM TTX was added to the ACSF to block Na+ currents. Cells were exposed to bath applied vehicle followed by increasing concentrations of nimodipine (100 nM to 10 μM). Aging neurons had prolonged calcium APs. 100 nM nimodipine decreased the calcium AP in aging neurons; 1 μM had little additional effect. In young neurons, 1 μM nimodipine had little or no effect on the calcium action potential, but 10 μM reduced it slightly. Nimodipine acted on the plateau phase of the calcium AP, which had a time course similar to the AHP and may underlie the enhanced AHP seen in these neurons. These data support the hypothesis that aging neurons have increased calcium influx that may contribute to the aging cell phenomenon, reduced calcium AP in an age- and concentration-dependent manner and may underlie its learning enhancement in aging animals. (Supported by ROI AG00187 and The Milne Institute).
600.9
REDUCED CONTROL OF MOTOR OUTPUT IN A HUMAN HAND MUSCLE OF OLDER SUBJECTS DURING SUBMAXIMAL CONTRACTIONS.
Aging is associated with a reduction in the number of motor units innervating a muscle, but with an increase in the innervation ratio of surviving motor units. The purpose of the study was to determine the effect of these age-related changes on the capability of subjects to control force during submaximal targets. Twenty-three healthy, neurologically normal human subjects (young: ages 20-37 years; old: ages 60-75 years) participated in the study. Motor unit activity was recorded from in dorsal interosseous muscle while the left index finger exerted an abduction force. The maximum voluntary contraction force recorded from first dorsal interosseous muscle while the left index finger exerted an abduction force. The maximum voluntary contraction force (MVC) was not different among subjects (29 vs 24 N for the young and old subjects, respectively). Subjects were instructed to hold the isometric force at four different levels (5%, 20%, 35% and 50% MVC) for 20 seconds. Elderly subjects displayed greater force fluctuations about each force level. The coefficient of variation about each force level (6.6%, 3.7%, 2.9%, 2.9% vs 11.0%, 5.0%, 4.2%, 3.9%) for the young and old subjects, respectively) was statistically greater for the older subjects. Moreover, the force exerted by single motor units (young: n=102; old: n=88) was found to be statistically greater in elderly subjects (29.1 vs 16.2 mN). However, the motor unit discharge characteristics were similar for the two groups of subjects. The presence of motor units with larger force amplitudes in elderly subjects may account for their decreased ability to control force.
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600.10
AGE DIFFERENCES IN VISUOMOTOR CONTROL. E.A. Roy*, T. Winchester and S. Black*. Department of Kinesiology, University of Waterloo, Waterloo and Sunnybrook Health Sciences Centre, Toronto, CANADA.
The movement characteristics of a simple aiming task were examined in two groups of right-handed subjects involving either younger (20-25 years) or older (60-75 years) adults. A WATSMART system was used to record positional coordinates of a marker placed on a stylus which was grasped in the right hand. Discrete pointing movements were made to a target of two different widths (12 mm or 57 mm) over two different amplitudes (150 mm or 300 mm) giving rise to indices of difficulty ranging from 2.4 to 5.65 bits. Subjects were instructed to move as quickly and accurately as possible. The results revealed that the overall movement time was greater for the older subjects for all conditions and reflected the relative difficulty of the task condition. Peak velocities achieved were considerably larger for the longer movements for both groups, with the younger subjects reaching higher peak velocities in all conditions. While time to peak velocity was similar between the groups, time after peak velocity differed significantly with the older subjects spending more time decelerating toward the target. The significance of these findings for understanding the effects of age on reaching is discussed with reference to current theories of motor control.

600.11
Mamillary bodies (MB) are hypothesized to play a critical role in declarative memory - a cognitive function that declines with age. In this study, age-related differences in the size of MB were examined using magnetic resonance (MR) imaging. The cross-sectional area of the MB was estimated from MR images of the brain in healthy volunteers and neurologically intact patients (N=82, age 18-78). The cross-sectional area of the tectum was used as a control region of interest (ROI). The area of the MB declined with age (r = -.51, p < .001); the area of the tectum did not (r = -.09, ns). Covarying skull size and sex did not alter the results. Supported by MSU Center for Applied Psychological Research.

600.12
The effects of aging on the size of selected cortical regions in 29 healthy volunteers and 54 patients with negative radiological findings who deceased due to dementia were examined using magnetic resonance imaging (MRI) scans. In both samples, similar patterns of cortical aging emerged. The size of sampled regions of association cortices (dorsolateral prefrontal and inferior parietal) correlated negatively with age, whereas no significant correlations between the size of primary somatosensory and visual cortices and age were found. In the first but not in the second sample, some of the correlations were attenuated after controlling statistically for skull size and sex. Overall, there were small but consistent trends for leftward asymmetry of the white matter, and rightward asymmetry of the grey matter. The results support the hypothesis of pathophysiology (selective aging) of association areas accompanied by relative sparing of sensory cortices. Supported by MSU Center for Applied Psychological Research.

600.13
The patients with Down’s syndrome (DS) invariably develop Alzheimer-type neuropathological changes at 40 years of age and older and display deficits in the ascending cholinergic and monoaminergic projections to patients with Alzheimer’s disease (AD). To address whether studying DS patients of different ages could serve as a model for progression of AD, we studied quantitative EEG and neurophysiological performance in an aging series of 31 DS patients, 36 patients with probable AD and age-matched controls. We found an age-related decrease of cortical functioning slowing of the EEG in DS patients aged from 20 to 60 years. EEG slowing, decrease of the peak frequency, was significantly related to Mini-Mental status scores, and visual, praxic and speech functions, and memory in the DS patients similarly as in the AD patients. Such correlations were not demonstrated for younger DS patients. The clinical and biopsychological and electrophysiological data to suggest that studying DS patients of different ages can serve as a model for progression of AD.

600.14
ENHANCED CORTISOL AND ACTH RESPONSES TO HYPERTONIC SALINE INFUSION IN OLDER HUMANS. E.R. Peskind*, M.A. Raskind, D. Winerup, M. Pascuali, D.J. Dobie, R.C. Veith, D.M. Dorsa, and C.W. Wilkinson. Dept. of Psychiatry, Univ. of Washington School of Medicine, and Seattle and American Lake VA GREEC, Seattle, WA 98195.
Hypertonic saline administration is a physiologic stimulus of the hypothalamic-pituitary-adrenal (HPA) axis. It increases plasma ACTH and cortisol in young humans and elevates CRH mRNA in the paraventricular nucleus of rats. This study addressed the hypothesis that the enhanced HPA axis responsivity demonstrated in old rats also occurs in normal aging humans and that it is altered in Alzheimer’s disease (AD). We administered a 90 minute hypertonic saline infusion (5% sodium chloride at 0.06 ml/kg/min) and a 90 minute saline infusion (0.9% sodium chloride at 0.06 ml/kg/min) to normal young men (n=11, age=29 ± 2 yrs), normal older men (n=7, age=63 ± 3 yrs), and otherwise healthy older men with AD (n=17, age=67 ± 2 yrs). Hypertonic saline produced substantial and equivalent increases in serum osmolality, serum sodium, plasma vasopressin, and plasma norepinephrine among groups. In contrast, the responses of plasma ACTH and plasma cortisol to hypertonic saline infusion as compared to placebo infusion were significantly greater in older normal and AD subjects than in young subjects. Cortisol and ACTH responses did not differ between older normal and AD subjects. These results suggest increased responsivity of the HPA axis in human aging. Supported by AG05136, AG08419, and the Dept. of Veterans Affairs.

The epidemiological association between traumatic head injury and Alzheimer's Disease, the detection of beta amyloid (IAP) deposition in the brains of professional boxers and reports of IAP deposition following acute head injury in the human post-mortem brain, lead us to ask if altered amyloid precursor protein (APP) localization and expression resulted after head injury in the rodent. The injury was delivered by a given weight dropped on the exposed dorsal surface overlying the parietal cortex from a height of 10 cm. After 24 hours dystrophic cells, neurites and fiber tracts confined to the injured area were intensely stained with N- and C-terminal APP antibodies, as well as with an antibody raised to a synthetic peptide containing the amino terminal portion of IAP. Specific staining was evident 4 hours after injury and persisted for up to one week. This aberrant APP staining correlated with the appearance of amino terminally truncated APP fragments on Western blots, clustered at ~22 Kd and ~70-80 Kd, also confined to the injured and penumbra regions. Gross alterations in total APP levels were not evident. The coincidence of increased APP staining in dystrophic cells and axons and the appearance of aberrant carboxyl terminal fragments confined to the injured regions suggest that altered APP processing, perhaps as a consequence of localized up regulation, occurs as an acute response to head trauma.


A synthetic 17-mer peptide corresponding to Ala319-Met335 of /ß/4-protein precursor (APP) has a neurotrophic effect on rat neuretoma cells (531-2,4) (4, 5). We evaluated the bioactivity of this peptide to reduce brain damage in a rabbit spinal cord reversible ischemia model (Zivin et al., Arch. Neurrol. 39:408-412, 1982). Ischemia of the caudal lumbar cord is produced by temporary occlusion of the abdominal aorta. Saline or peptide (200, 500 or 1000 nm) was administered i.t. 20 minutes prior to ischemia and once daily for three days thereafter. Neurologic outcome was evaluated after four days. Durations of ischemia encompassing all grades of neurologic function were included. The 500 nm dose significantly reduced neurologic damage. The average ischemia duration necessary to produce permanent neurologic damage increased from 30.1 ± 1.77 min in controls to 41.4 ± 4.7 min in the 500 nm group. The 200 nm dose produced a nonsignificant trend toward reduced neurologic damage. Our results demonstrate that this 17-mer peptide is capable of reducing neurologic damage in vivo in a model of CNS ischemia, a finding consistent with a neurotrophic effect of APP.

MICE TRANSGENIC FOR HUMAN /ß/AMYLOID PROTEIN ARE IMPAIRED IN SPATIAL, BUT NOT CUSED LEARNING. G. Tirado-Santillo1, J. Naibert2, R. J. Dijkstra1, B. Smith1, F. J. Mendelsohn*1, L. Haiss2, W. J. Edward2, D. Baltimore2, G. H. Martin2, M. A. Lambrecht1, J. H. Donahue2, G. R. Bell2, and J. E. Craig2. Departments of Physiology and Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada.

Brain deposition of /ß/amyloid is an early marker of Alzheimer's disease. Mice (C57BL/6, 8 months old) transgenic for a human /ß/amyloid protein fragment were compared to normal litter mates in spatial and nonspatial learning tasks in the Morris water maze. A cDNA fragment including amino acids 591-695, spanning the amyloid-forming and C-terminus portion of the /ß/amyloid precursor protein, was cloned into the first exon of the human neurofibrilamyloid /ß/ gene, under the transcriptional control of the NF-L promoter. Northern analysis showed 3 of 7 transgenic lines expressed human /ß/-amyloid transcripts in brain tissue. Spatial and non-spatial learning were tested using standard procedures in the Morris water maze task (n: transgenic = 4, control = 7). Behavior was tested blind to the genetic heritage of the mice. The Johns Hopkins Morris water maze task showed a time trend: the higher the temperature was reduced from 25°C to 20°C, the more the behavior was impaired. Two measures of learning and performance have been analyzed statistically thus far: escape latency and the number of trials correct (reaching the platform before the end of a trial). Transgenic mice were impaired relative to their litter mates in spatial learning in both measures and only 25% of the control cells remain attached. Transfected cells at the light microscopy level showed increased cell clumping. These data suggest that overexpression of the /ß/-amyloid terminal region of APP may alter both cell-substrate and cell-cell adhesion.

The characterization of Alzheimer's Disease (AD) is the deposition of β-amyloid into neuritic- and cerebralvascular plaques. This pathology has been implicated in the manifestation of the clinical syndrome of AD. β-amyloid is a 42-amino acid peptide which is derived from a larger precursor protein (APP). Alternate exons usage results in the generation of at least three APP forms (695, 751, and 770 amino acids) each of which contains the sequence for β-amyloid. It has been suggested that either overexpression of APP, disruption of the tissue specific balance of the APP forms, or abnormal processing of the precursor protein may result in the deposition of β-amyloid. The major single nucleotide mutations in APP have been reported in some Familial AD pedigrees and in Hereditary (Dutch) Cerebral Hemorrhage with Amyloidosis (HCHWA-D). In order to test the causal relationship of the above hypotheses on plaque formation as part of the etiology in AD, we have used transgene based expression of the human APP in transgenic mice. We have also created the construction of the vectors and preliminary molecular biological analysis will be presented.

601.9 EVIDENCE THAT BRAIN SYNAPTIC MEMBRANES CONTAIN A RECEPTOR FOR THE ALZHEIMERAMYLOID PEPTIDE. B.A. Bahr, B. Abi-Saab, S. Alimardani, S. Vier, and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92697-3800.

Alzheimer's disease neurodegeneration is associated with deposition of the 39- to 43-amino acid amyloid β-amyloid in neuritic and cerebral synaptic vascular plaques. The precursor protein for Aβ (APP) has been suggested to be a specialized adhesion molecule necessary for synaptic formation and function (Brenn et al. J. Neurosci. Res. 29:1011, 1991; Schubert et al., Science 249:84, 1990; et al.,) such proteins have been identified (Bahr & Lynch, Biochemistry, 1990, 31:171, 1992). Furthermore, expression of APPs has been shown to be strongly linked to the development of synaptic plasticity and synaptic loss (Maeda et al., Neuron. Lett. 100:24, 1989). Accordingly, rat synaptic membranes (SPMs) were seeded onto microtitre wells coated with portions of APP or extracellular matrix proteins in order to test for homotypic and/or heterotypic receptors that recognize synaptic matrix proteins. Subsequent assays using antibodies to the neural terminal marker synaptophysin demonstrated that recombinant SPMs bound to neomycin, fibronectin, enaetin, and to a lesser extent laminin, fibrinogen, and collagen. In comparison, it appears that the greatest amount of SPM attachment was directed to neuritic cytoskeletal fragments of APP including bAPP-CJD which spans the Aβ and cytoplasmic, membrane spanning, and carboxyterminal fragments of APP, (Aβ40 or Aβ42) and to a degree i) Aβ40. No SPMs bound to βAPP-16, βAPP, αS, or substance P which has a similar amino acid sequence to βAPP-35. The βAPP-25-35 binding had the following regional selectivity: hippocampus = neocortex > striatum > cerebellum > neostriatum > thalamus = brain stem = olfactory bulb. The binding was inhibited by 100 μM Aβ41-40 and by the adhesion blocker GHRGDP (EC50 ~ 61 ± 2 μM), but not by 1 mM Aβ1-16, 100 μM substance P, 100 μM physteine, 10 mM GRADDP, or 3 mM REDV. Membrane-SPM attachment, however, was blocked by REDV (EC50 ~ 30 μM) but not by Aβ1-40 or Aβ2-35. This data suggest that at least two receptor types are involved in synaptic matrix recognition, perhaps one of which plays an active role in synaptic pathogenesis (Supported by the HSFP).


The mechanisms involved in β-amyloid deposition in Alzheimer's disease are largely unknown. We report here in part involve overexpression of the β-amyloid precursor protein (βAPP). The promoter region of this gene contains two upstream sequences homologous to the transcription factor activator protein 1-AP1) recognition sites. Activation of protein kinase C (PKC) leads to the induction of Fos and Jun (major constituents of AP1), increased AP1 DNA binding activity, and transcriptional activation of AP1 responsive genes. To determine whether PKC activation alters βAPP RNA levels, we measured βAPP mRNA levels and transcriptional activation of a 500 bp βAPP promoter-ketilerase reporter gene construct (βAPP-LUC) in a human glioma (U37) cell line (123HJ1) cell line co-expressing PKC with or without cholera toxin PMA treatment. PKC activation leads to a 4-fold increase in βAPP mRNA levels. In transient transfection experiments the βAPP-LUC is transcriptionally activated by PMA as well as by co-expressed transcriptional PKC. We used gel mobility shift assays to determine if PMA induces protein DNA binding to either of two oligonucleotides corresponding to the putative AP-1 sites. The upstream AP1 site effectively binds to PMA stimulated cells whereas the downstream AP1 site fails to exhibit binding activity. To directly establish a role for Fos and Jun in transcriptional regulation of the βAPP gene, cells were co-transfected with a βAPP-LUC reporter construct together with Fos and Jun expression plasmids. Surprisingly, the βAPP promoter is transactivated by coexpression of Jun while Fos appears to repress transcriptional activation. We conclude that Fos and Jun play a pivotal role in the expression of the βAPP gene and that PKC and induction of Jun/AP1 which binds to the upstream AP1 site to activate transcription.
NEURODEGENERATION INDUCED BY B-AMYLOID PEPTIDES IN VITRO MAY FOLLOW A APOPTOTIC PATHWAY. D. Copen1, D.T. Loo, C.I. Fink, A.J. Wiedenmann, and C.W. Coman, Irvine Research Unit, University of California, Irvine, CA 92717 USA. In the nervous system cell death may occur through two distinct processes: necrosis and apoptosis. Necrotic cell death, resulting from acute injury, is characterized by cell swelling and lysing; conversely, apoptotic cell death is characterized by cell shrinkage and release of membrane-bound bodies. The factors that trigger apoptosis in the nervous system have not been proposed to activate an endogenous program of cellular self-destruction, yet they remain poorly defined. In Alzheimer’s disease, β-amyloid is hypothesized to contribute to neuronal loss. We have reported that aggregated β-amyloid peptides (βAPs) can be neurotoxic in vitro. Here, we analyze morphological and biochemical events associated with βAP-induced neurodegeneration to understand the mechanism by which treated neurons die. Degenerating neurons, observed under phase-contrast microscopy during a 24 hr exposure to βAPs, exhibited condensed cell bodies with pyknotic nuclei. The degenerative events proceeded asynchronously and affected a significant portion of the neuronal population. At 24 hr a disparity between the number of degenerating neurons and the release of lactate dehydrogenase was observed, suggesting that cell lysis is not the initial event in the degenerative process. A breakdown of cellular DNA into oligonucleosome-length fragments is often associated with apoptosis. Using agarose gel electrophoresis, we found that βAP-treated cultures exhibited a regularly spaced “ladder” of DNA fragments. Aurin-tricarboxylic acid, an inhibitor of nucleases in vitro, suppressed the DNA fragmentation and delayed neuronal lysis when added to the cultures simultaneously with the pep­tide. Unreated cultures exhibited measurable DNA fragmentation, likely resulting from normally occurring cell death in vitro, but it was significantly less than in βAP-treated cultures. Our study suggests that neurons exposed to βAPs degenerate in a manner consistent with morphological and biochemical changes characteristic of apoptosis, perhaps as a result of accelerated normal cell death in vitro.

α-antichymotrypsin (ACT), a serine protease inhibitor, was found to be one of the components of senile plaque amyloid (Abraham et al., Cell 52:487, 1988). In situ hybridization studies showed that ACT mRNA was localized in reactive astrocytes around senile plaques, indicating that reactive astrocytes were the origin of ACT (Pasterнак et al., Am. J. Pathol. 135:827, 1989; Koo et al., Neurobiol. Aging 12:449, 1991). To confirm this possibility, we studied the synthesis and secretion of ACT using primary murine astrocyte cultures obtained from one day old neonatal brains. We stained cultured astrocytes with a polyclonal antibody to human ACT (Dako) which revealed cytoplasmic granular labeling consistent with compartmentalization in secretory granules. This staining was completely abolished by preincubation of the antibodies with unlabeled ACT. Immunofluorescent double labeling revealed that ACT co-localized with ubiquitin normally caused by a hypoglycemic insult. Since hypoglycemic damage. B. Cheng1*, V. L. Smith-Swintosky1 I. Ritter2, S. L. Smith-Swintosky2, L. Lieberburg*, R. E. Rydell2 and M. P. Mattson*. 1Department of Anatomy & Neurobiol., Univ. of Kentucky, Lexington, KY 40536; 2Athena Neurosciences, S. San Francisco, CA 94080.

We have established a method for long-term culture of human cortical neurons. Chronic administration of 4 μM of the β amyloid peptide β1-40 resulted in progressive neuronal degeneration. Immunocytochemical analysis showed that β1-40 gradually accumulated specifically on the neuronal soma as the incubation time increased, forming compacted deposits that contain the cell bodies of degenerating neurons and are surrounded by dystrophic neurites. Amyloid deposition was not observed or increased in frequency with the peptide, indicating that reactive astrocytes synthesize and secrete an active ACT-like protein. ACT, secreted by reactive astrocytes, may disturb the balance between the processing of the β amyloid precursor protein. (Supported by NIH AG-09905).

602.3 BETA AMYLOID DEPOSITION AND NEURONAL DEGENERATION IN CULTURED HUMAN CORTICAL NEURONS. J. Bucigrop* and B.A. Yanker, Dept. of Neurology, Harvard Medical School and The Children’s Hospital, Boston, MA 02115.

We have established a method for long-term culture of human cortical neurons. Chronic administration of 4 μM of the β amyloid peptide β1-40 resulted in progressive neuronal degeneration. Immunocytochemical analysis showed that β1-40 gradually accumulated specifically on the neuronal soma as the incubation time increased, forming compacted deposits that contain the cell bodies of degenerating neurons and are surrounded by dystrophic neurites. Amyloid deposition was not observed or increased in frequency with the peptide, indicating that reactive astrocytes synthesize and secrete an active ACT-like protein. ACT, secreted by reactive astrocytes, may disturb the balance between the processing of the β amyloid precursor protein. (Supported by NIH AG-09905).

602.4 SUBSTRATE-BOUND BETA-AMYLOID PEPTIDE: NEUROTROPHIC EFFECT ON PRIMARY CEREBRAL CORTEXAL NEURONS IN VITRO. J.R. Wijglo*, M.D. Dorhy, J. Silver, and R.C.A. Fredericksen. Gliatech, Inc., Cleveland, OH 44122 & Case Western Reserve Univ., Cleveland, OH 44106.

Beta-amyloid peptide (βAP), when dissolved in culture medium, has been reported to be neurotoxic to primary neurons. (Yanker et al., 1989, Science 245:417). However, β-amyloid in Alzheimer's disease is primarily found as insoluble plaques. Therefore, we have examined the effect of a sub-neurotoxic concentration of βAP on primary cortical neurons in vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro. A small volume of βAP was applied to cultures of primary cortical neurons. In vitro.


In the accompanying abstract (Mattson et al.) we reported that secreted forms of amyloid precursor protein (APP) exert a potent calcium-lowering effect in cultured rat hippocampal and human cortical neurons. Since there is increasing evidence that APP can also act as a neurotrophic/neuromodulatory molecule to both APPS695 and APPS 751 (aas. 444-592 of APP695) abolished the function of APPs and (2) liberating 8-amyloid peptide which may enhance neuronal vulnerability to ischemic/excitotoxic insults. (Supported by NIH AG-09905).
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602.7


Induction of amyloid precursor protein mRNA in rat cerebral cortex has recently been reported (Abe et al., J. Cereb. Blood Flow Met., 11, 1991). In order to determine if expression of APP itself is N-terminal and its cellular origin, we performed immunocytochemistry using antibodies to various epitopes of APP on tissue from rats subjected to permanent middle cerebral artery occlusion (MCAO). Spontaneously hypertensive rats were anesthetized with isoflurane and the middle cerebral artery was occluded by a MCAO. Ten micrograms of adenosine 3'5'-cyclic monophosphate (cAMP) was dissolved in ultrapure water (10 mg/ml) and allowed to aggregate for at least 32 days in vitro (DIV) are associated with changes in cell composition and organization undergone by primary cultures of rat cerebral cortex between 7 and 32 days in vitro (DIV).

602.9

ß-AMYLOID CONTAINING PEPTIDES PRODUCED IN NEWBORN RAT CEREBRAL CORTEX PRIMARY CULTURES. Xue R. LeBlanc A C. LaSala D. Clifton PC. Alzheimer's and Dementia Research, Lederle Laboratories, American Cyanamid Company, Pearl River, NY, 10965.

ß-amyloid protein (ß-AP) is a peptide that abnormally deposits in diseases such as Alzheimer's disease (AD). Recent evidence has suggested that the accumulation of this peptide may result in neuronal death and the formation of neuritophied tangles. Toxic effects of aggregated ß-AP have been demonstrated, and several studies have suggested that these toxic effects can be reversed by exposure to synthetic amyloid peptides. Further, it has been shown that aggregation alters toxicity. In this study, we examined effects of aggregated ß-AP on primary cultures of newborn rat hippocampal neurons. Synthesis of ß-AP was homologous to amino acids 1-40 (ß1-40) and 25-35 (ß25-35) of ß-AP. Mice treated with ß25-35 and ß1-40 have significantly decreased memory compared to control mice. In this study, we examined the effects of synthetic ß-APs on rat microglia in culture. The major effects reported to be influenced by the state of peptide aggregation. We have examined the effects of synthetic ß-APs on rat microglia in culture. Two different ß-APs were used: ß1-42, a full-length homolog of the ß-amyloid protein deposited into senile plaques, and ß25-35, an active fragment of ß-amyloid protein that exhibits both aggregative and neurodegenerative properties. Cultures treated with both ß1-42 and ß25-35 contained reactive gliosis, indicating the presence of aggregated ß-APs. Within hours after their exposure to purified cultures of ramified microglia, ß25-35 had induced severe beading of processes; often processes appeared discontinuous and with diminished bright-field microscopy from 1-42 also induced this morphological effect, although often with less intensity than ß25-35.

These observations indicate that aggregated ß-APs, in addition to their reported neurodegenerative properties, also induce morphological changes in microglia in vitro.

602.12


Microglia are often associated with senile plaques, a pathological hallmark of Alzheimer's disease. The insoluble core of a senile plaque consists largely of aggregated ß-amyloid protein. Synthetic ß-amyloid peptides (ßAPs) can induce neurodegeneration in vitro, an effect reported to be influenced by the state of peptide aggregation. We have examined the effects of synthetic ßAPs on rat microglia in culture. Two different ßAPs were used: ß1-42, a full-length homolog of the ß-amyloid protein deposited into senile plaques, and ß25-35, an active fragment of ß-amyloid protein that exhibits both aggregative and neurodegenerative properties. Cultures treated with both ß1-42 and ß25-35 contained visible precipitants, indicating the presence of aggregated ß-AP.

Within hours after their exposure to purified cultures of ramified microglia, ß25-35 had induced severe beading of processes; often processes appeared discontinuous under light microscopy. ß1-42 also induced this morphological effect, although often with less intensity than ß25-35.

These observations indicate that aggregated ß-APs, in addition to their reported neurodegenerative properties, also induce morphological changes in microglia in vitro.

AMYLOID P component (APC) is a globular glycoprotein associated with the amyloid proteins that accumulate in virtually all amyloidoses, including Alzheimer disease (AD). This study continues our analyses of APC's association with the lesions of AD, particularly "preamyloid" and neurofibrillary tangles (NFT). Autoradiographs from AD and normal-AD cases were fixed in mixed aldehydes and immunocytochemically labeled with a polyclonal antibody against APC (Dako-Patts). Reaction product was visualized with the avidin-biotin-peroxidase technique (Vectorlum), using diaminobenzidine as chromagen. In all cases, regional foci of labeled neurons, astrocytes, microglia, and/or oligodendrocytes were seen; thus APC accumulation is not specific to AD. In AD, the cytoplasm of intracellular-NFT-laden neurons was labeled, while the extracellular (E)-NFT themselves were darkly stained. Some plaques resembling "preamyloid" deposits were dispersed through the neuropil. More classical-appearing accumulations contained immunolabeled knob-like structures that, at the light microscopic level, appeared to be dystrophic neurons. However, ultrastructural analyses revealed that dystrophic neurons were never immunolabeled. Hence, these accumulations may not be classic plaques, but rather diffuse accumulations of APC around E-NFT.

Amyloid B-protein is a major component of senile plaques found in brains of patients with Alzheimer's disease. It is hypothesized that this small protein is a pathological degradation product of the amyloid precursor protein (APP). We have found that both full length and truncated, secreted APP exist as the core protein of a chondroitin sulfate proteoglycan (CSPG). The secreted APP CSPG in C6 cell culture medium was partially purified by ion-exchange and gel filtration chromatography. Western blot analysis revealed a diffuse band (ca 140k-250k). This material was detected by several antibodies which specifically recognize different regions of APP. Chondroitinase AC or ABC-treatment completely eliminated this high molecular weight band with a concomitant increase in the APP band (120kD). The digested product reacted with an anti-stub monoclonal antibody which recognizes 4-sulfated disaccharide. Heparinase or heparitinase did not have any effect on this PG. The N-terminal sequence of this CSPG core protein matched with that of APP. Denaturing analysis showed that about 90% of the secreted APP can occur in the CSPG form. CSPG form of full length APP was also detected on the cell surface. The close proximity of two concomitant CS attachment sites to both the N-terminus of the amyloid B-protein and the secretase cleavage site, suggests that the CS chains may affect the APP processing and/or production of the amyloid B-protein.

PHOSPHORYLATION OF β42 AMYLOID PRECURSOR PROTEIN (APP) IN VIVO. E. Shapiro1,2, L. McConlogue1, K.L. Eide1, T.R. Soderling2. 1Department of Pathology and 2Vollum Institute, Oregon Health Sciences University, Portland, OR 97201 and 3Athena Neurosciences, South San Francisco, CA 94080.

We wanted to determine the protein kinase(s) which phosphorylate APP, the site(s) of APP phosphorylation, and whether changes in the biochemistry of APP might be associated with APP phosphorylation. Cultured S9 insect cells, infected for 48h with recombinant APPβ42-baculovirus, were labeled with [32P]orthophosphate and immunoprecipitated with a polyclonal anti-APP590-695 antibody (C-terminal-immunoreactive). Under basal conditions, APP was phosphorylated on Ser only. PKC(a) stimulated the 32P-phosphorylation of full-length APP (200kDa) and of a ~10kDa C-terminal APP fragment (10-15kDa) in S9 cells co-expressing PKC and APP. The same site in the cytoplasmic domain of APP was phosphorylated by PKC in full-length APP and in the in situ-generated C-terminal fragment, as indicated by the presence of the same PKC-stimulated tryptic phosphopeptide in both APP species. PKC stimulated 32P incorporation into Pher only. One possible explanation for the large relative increase in 32P labeling of the C-terminal fragment is that PKC stimulates the accumulation of this fragment. Experiments to characterize the C-terminal fragment, the precise PKC phosphorylation site, and the mechanism of increased 32P incorporation into this APP C-terminal fragment are ongoing.

These studies demonstrate that PKC phosphorylates APP and that APP phosphorylation may regulate the processing of APP in vivo.

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β-AMYLOID-INDUCED NEURODEGENERATION IN VITRO: RELATIONSHIP TO PEPTIDE AGGREGATION. C.L. Pika*, D. Burdis1, A.J. Walencewicz1, C.G. Glaab2 and C.W. Cotman1. 1Irvine Research Unit in Brain Aging and 2Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717 USA.

The β-amyloid protein is present as an insoluble aggregate within senile plaques, the definitive lesions of Alzheimer's disease (AD). We have demonstrated previously that β1-42, a full-length synthetic β-amyloid peptide (βAP), readily forms aggregates in vitro and is neurotoxic to cultured hippocampal neurons when in an aggregated state. Here we have examined an overlapping series of βAPs to investigate further the structure-function relationship between βAP aggregation and neurotoxic properties of β-amyloid protein. βAPs were studied both after initial solubilization in water and after a 7-day incubation of these solutions. Aggregation of βAP, the site(s) of APP phosphorylation, and whether changes in the biochemistry of APP might be associated with APP phosphorylation. Cultured S9 insect cells, infected for 48h with recombinant APPβ42-baculovirus, were labeled with [32P]orthophosphate and immunoprecipitated with a polyclonal anti-APP590-695 antibody (C-terminal-immunoreactive). Under basal conditions, APP was phosphorylated on Ser only. PKC(a) stimulated the 32P-phosphorylation of full-length APP (200kDa) and of a ~10kDa C-terminal APP fragment (10-15kDa) in S9 cells co-expressing PKC and APP. The same site in the cytoplasmic domain of APP was phosphorylated by PKC in full-length APP and in the in situ-generated C-terminal fragment, as indicated by the presence of the same PKC-stimulated tryptic phosphopeptide in both APP species. PKC stimulated 32P incorporation into Pher only. One possible explanation for the large relative increase in 32P labeling of the C-terminal fragment is that PKC stimulates the accumulation of this fragment. Experiments to characterize the C-terminal fragment, the precise PKC phosphorylation site, and the mechanism of increased 32P incorporation into this APP C-terminal fragment are ongoing.

These studies demonstrate that PKC phosphorylates APP and that APP phosphorylation may regulate the processing of APP in vivo.

β-AMYLOID-INDUCED NEURODEGENERATION IN VITRO: RELATIONSHIP TO PEPTIDE AGGREGATION. C.L. Pika*, D. Burdis1, A.J. Walencewicz1, C.G. Glaab2 and C.W. Cotman1. 1Irvine Research Unit in Brain Aging and 2Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717 USA.

The β-amyloid protein is present as an insoluble aggregate within senile plaques, the definitive lesions of Alzheimer's disease (AD). We have demonstrated previously that β1-42, a full-length synthetic β-amyloid peptide (βAP), readily forms aggregates in vitro and is neurotoxic to cultured hippocampal neurons when in an aggregated state. Here we have examined an overlapping series of βAPs to investigate further the structure-function relationship between βAP aggregation and neurotoxic properties of β-amyloid protein. βAPs were studied both after initial solubilization in water and after a 7-day incubation of these solutions. Aggregation of βAP, the site(s) of APP phosphorylation, and whether changes in the biochemistry of APP might be associated with APP phosphorylation. Cultured S9 insect cells, infected for 48h with recombinant APPβ42-baculovirus, were labeled with [32P]orthophosphate and immunoprecipitated with a polyclonal anti-APP590-695 antibody (C-terminal-immunoreactive). Under basal conditions, APP was phosphorylated on Ser only. PKC(a) stimulated the 32P-phosphorylation of full-length APP (200kDa) and of a ~10kDa C-terminal APP fragment (10-15kDa) in S9 cells co-expressing PKC and APP. The same site in the cytoplasmic domain of APP was phosphorylated by PKC in full-length APP and in the in situ-generated C-terminal fragment, as indicated by the presence of the same PKC-stimulated tryptic phosphopeptide in both APP species. PKC stimulated 32P incorporation into Pher only. One possible explanation for the large relative increase in 32P labeling of the C-terminal fragment is that PKC stimulates the accumulation of this fragment. Experiments to characterize the C-terminal fragment, the precise PKC phosphorylation site, and the mechanism of increased 32P incorporation into this APP C-terminal fragment are ongoing.

These studies demonstrate that PKC phosphorylates APP and that APP phosphorylation may regulate the processing of APP in vivo.

Weaver is an autosomal recessive mutation in mice that affects the differentiation and vitality of several classes of neurons. In the midbrain, many of the dopamine-containing cells of the substantia nigra fail to differentiate normally and die after an embryonic critical period. This is a report of our preliminary results of the fate of these neurons placed in culture.

For these experiments, the ventral mesencephalon and the striatum were removed from the brains of 7-day-old pups that were identified as being either homozygous weaver or homozygous normal mice by the appearance of the cerebellum. Disassociated cells from midbrain and striatum of the same mouse were plated together with poly-L-lysine, laminin and fibronectin. The coverslips were placed in 24-well plates with modified DMEM and serum.

After 4 days in culture, the cells were fixed and stained for the presence of tyrosine hydroxylase (TH) to identify the dopamine-containing populations. Both weaver and wild-type TH-positive neurons displayed both fusiform and polygonal morphology. In cultures of weaver, the TH-positive fusiform cells outnumbered the polygonal types by a ratio of 4 to 1; whereas polygonal forms outnumbered fusiform by a ratio of approximately 3 to 1 in the wild-type. Pyknotic nuclei were abundant in the weaver cultures and scarce in the wild-type indicating that cell death took place in the weaver cultures during this period at a greater rate than in the wild-type cultures.

This culture system appears to reflect the early cell death of the weaver's dopamine-containing neurons that takes place in vivo. These cultures may serve as a useful assay for conditions that influence the development and long-term survival of dopamine-containing neurons. NIH NS20181

603.3 NMDA-MEDIATED GLUTAMATE NEUROTOXICITY IN MESENCEPHALIC DOPAMINE NEURONS IN CULTURE. S. Kishiuchi and F.U. Kita* Dept. of Neurology, Univ. of British Columbia, Vancouver, Canada

Neurotoxic action of excitatory amino acids has been considered as one of the causes of the neuronal loss in neurodegenerative diseases that include Parkinson disease. In this study, we investigated the neurotoxic effect of glutamate in dopaminergic neurons grown in culture. Dissociated cell cultures were prepared from 13 day fetal mouse mesencephalon, while 10-12 day old cultures were exposed to glutamate for 10 minutes, and 24 hours later cultures were evaluated for glutamate neurotoxicity by tyrosine hydroxylase (TH) and MAP2 immunostaining and by dopamine uptake assay. In the glutamate-exposed cultures, the number of TH-positive and MAP2-positive neurons and the level of dopamine uptake were decreased to 30-50% of the control. Glutamate neurotoxicity was completely blocked by MEMO1, an NMDA receptor antagonist, and was reduced by magnesium ions. Phorbol ester and gangliosides (CM1 and GT1b) were also found to block glutamate neurotoxicity, suggesting that protein kinase C translocation and activation might be important steps in the pathomechanism of glutamate neurotoxicity.

603.5 DEPRENYL AND CLORGYLINE SUPPRESS HYDROXYL RADICAL GENERATION DURING DOPAMINE OVERFLOW ELUCIDATED BY L-METHYL-MPTP. S.-J. Huang, C.C. Chishti and D.L. Murphy. Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

We have recently reported the use of in vivo intracranial microdialysis perfusion of salicylate to assess hydroxyl radicals (+OH) formation in the brain. The (+OH) adducts of salicylate were measured by HPLC-EC. Intratransient dialysate of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) enhanced +OH formation in vivo. Further, +OH formation may be due to antioxidation of the released DA in the basal ganglia where high levels of iron are present. (+OH) may also be formed during the dopamine of released DA and the oxidation of 2'-methyl-MPTP by A and B type monoamine oxidase. This data leads to new working hypothesis that monoamine oxidase inhibitors may protect against MPTP-induced selective DA neurotoxicity by suppressing the generation of cytotoxic (+OH) radicals in the iron-enriched basal ganglia.


Normal neuronal cell death either in vivo, during embryonic development or in vitro in cell culture models has been reported to be an active process, dependent on gene expression and on its specific synthetic (Ellis et al., Nature 363, 603, 1993). This is a report of our preliminary results of the gene expression on gene expression of the ionophore A23187 in differentially PC12 cells, i.e., a type of cells which exhibits apoptosis similar to dopaminergic neurons.

In PC12 cells grown for 8-12 days in presence of NGF25g/ml and serum, treatment with low concentrations of the calcium ionophore A23187 (3-134nM) for 24hrs produced a sustained increase in dopamine (DA) efflux from the cells. In the glutamate-exposed cultures, the number of lipid-seeking bodies as characterized by the pyramidal exclusion criterion. Accordingly, (+OH) dopaminergic uptake, an index of cellular integrity was impaired in this range of concentrations. Neuronal differentiation could be resumed upon withdrawal of A23187 in the presence of NGF. Widespread cell degeneration occurred at higher concentrations only ( >30nM). Electron microscopical studies revealed morphological changes in the cytoplasm (formation of lipid droplets; mitochondrial shrinkage; reticulum vacuolization) but no obvious nuclear alterations such as chromatina segregation. In addition, DNA fragmentation could not be detected on agarose gels suggesting that in this culture model, nuclear activation is not a prerequisite for cell death. Other factors such as Ca2+ activated proteases or deregulation of certain genes that lead to irreversible damage are being studied.

603.4 MK-801 DOES NOT PREVENT DOPAMINERGIC CELL DEATH INDUCED BY MPP+ IN RAT MESENCEPHALIC CULTURES. Y. Aycid, B. Zalc* and P.P. Michel. INSERM U289 et U134, Hôpital de la Salpêtrière, 75013 Paris, France.

Neuroprotective effects of MK-801 were tested in a culture model reproducing the selective degenerative process in dopaminergic neurons in parkinsonian brains (Michel et al., J. Neurochem., 54, 1102, 1990). Dissociated mesencephlic cells derived from embryonic rat brains were exposed to the 1-methyl-4-phenylpyridinium ion (MPP+), the active metabolite of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPP+ at low concentrations (3 and 10μM) produced selective and dose-dependent toxic effects towards dopaminergic neurons as quantified by the loss of tyrosine hydroxylase (TH) neurons and the loss of [3H]-DA uptake whereas non dopaminergic cell types remained unaffected. MK-801 at 3 and 10μM did not rescue degenerative neuronal death. MAP2, i.e., the highest concentration that is not toxic by itself in this culture system, MK-801 was also ineffective. Additionally, degree of dopaminergic damage was not reduced when repeated additions of the glutamate antagonist were performed during exposure to MPP+ or when mesencephalic cultures were left after intoxication for several days in a culture medium still supplemented with MK-801 but lacking the toxin. Furthermore, in control cultures, MK-801 did not affect the uptake of [3H]-DA significantly, thereby suggesting that this compound does not interfere with the accumulation of MPP+ into dopaminergic nerve terminals. At higher concentrations tested (100μM), MPP+ produced a non selective destruction of all cultured cells as characterized by the loss of the number of pyramidal exclusion bodies and the loss of [3H]-GABA uptake. In these conditions, MK-801 was also found ineffective. Altogether these results indicate that MPP+ neurotoxic effects are not related to an excitotoxic process and/or that MK-801 does not interfere with MPP+ toxic scenario. Therefore, neuroprotective agents may prove not to be effective in preventing dopaminergic cell degeneration in Parkinson's disease.

603.6 A METHOD FOR MEASURING Dopamine-ProTEIN CONJUGATES AS AN INDEX OF Dopamine OxidATION. M. Zigmund and T.G. Hastings. Dept. of Cellular and Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Dopamine (DA) and free radicals are implicated in the induction of striatal damage associated with certain neurotoxic events. In the presence of oxygen, DA oxidizes to free radical species and DA quinones. The quinones may attack cellular proteins resulting in potentially complex alterations in cellular function. To establish a method for measuring DA-protein conjugates as an index of DA oxidation, we incubated striatal slices with [3H]-DA under various buffer conditions and determined the amount of radioactivity bound to acid-precipitated protein. The amount of tritium bound to protein was greatly influenced by the concentration of reducing agent (ascorbate or glutathione) present in the incubation buffer. After a 1 h incubation at 37°C, there was a 3.4-fold increase in the amount of tritium bound to protein in a Krebs-bicarbonate buffer with ascorbate, as compared with the same buffer containing 0.85 mM ascorbate (2.52 ± 0.43 pmol/mg prot and 0.74 ± 0.04 pmol/mg prot, respectively). The difference increased to 6.6-fold after 120 min. The amount of tritium bound to protein was reduced by 90% in the presence of 10 μM glutathione. The amount of tritium bound to protein at 100 μM [3H]-DA was isolated by HPLC in a 1 to 2.5 ratio. These findings suggest that: 1) the oxidation products of DA and DA metabolites are binding to protein; 2) binding occurs by covalent interactions with protein sulfhydryl groups; and 3) DA oxidation associated with cytotoxicity would be strongly influenced by cellular maintenance of antioxidant capacities. (Supported in part by USPHS grants NS19608, MH00058, and NS0076.)
USE OF SALICYLATE TO TRAP HYDROXYL RADICALS IN RAT BRAIN: A METHODOLOGICAL STUDY

Formulation of free radicals in brain has been implicated in many neurodegenerative processes, including those induced by ischemia, excitatory amino acids, and amphetamines. It has been reported that salicylate can react with hydroxyl radicals to form the stable adducts 2,3- and 2,5-dihydroxybenzoic acid (DHBA) (Floyd et al., J Free Rad. Biol. Med., 1986). Tissue levels of these adducts can be determined by HPLC with electrochemical detection. We assessed the practicality of this method and determined a paradigm for estimating the content of hydroxyl radicals in rat brain. Following its systemic administration (100 mg/kg, i.p.), salicylate levels peaked at approximately 150 nmol/g tissue at 2 hrs and then declined with a half time of 4 hr in several brain areas, including striatum, frontal cortex, and hippocampus. The major adduct of salicylate formed in vivo was 2,5-DHB.A, which reached a peak of approximately 250 pmol/g tissue (2 hr) in different areas. DHBA levels were not affected by the addition of the reducing agent ascorbate (0.5 mM) and/or the iron chelator DTPA (1 mM) during tissue homogenization (sonication at 100 mg/ml in 0.2 M perchlorate). Freezing the tissue (−70°C) also had no effect. We are using this method to examine formation of free radicals in vivo under several conditions associated with an increase in DA availability, such as methamphetamine exposure (see Giovanni, Soc. Neurosci. Abst., 1992) and L-DOXP treatment. Our data demonstrate the usefulness of this technique to trap free radicals in vivo to quantify these highly reactive and short lived oxygen species. (Supported in part by NIH grants NS19608, MH00058, and NS09076.)

THE HYDROGEN TRANSFER MECHANISM. J. D. Adams* and L. d°opamine, and related compounds have been reported. Numerous peroxidase-type enzymes are capable of catalyzing the oxidation of DA to reactive quinones, however, these enzymes are not found in brain. Prostaglandins (PG) synthesize an arachidonic acid metabolizing enzyme, prominent in brain and has peroxidase activity that requires reducing cosubstrates such as catechol-containing compounds. As a result of this reaction, DA may be co-oxidized to DA quinones and free radical semiquinones. To investigate this possibility, we examined whether purified PG synthase would catalyze the oxidation of DA in vitro. Utilizing spectrophotometric analysis, we measured the formation of the PG synthase-catalyzed oxidation of DA resulted in the binding of DA to protein which was identified as cytosine-DA conjugates. The amount of cytosine-DA isolated from protein was increased 2-4 fold in the presence of PG synthase, using either substrate. The identification of cytosine-DA indicates that the enzyme catalyzes the co-oxidation of DA to quinones which are capable of reacting with nucleophilic thiols on proteins. DA-oxidation catalyzed by PG synthase represents potential mechanisms for protein oxidation and cytotoxicity. (Supported in part by USPHS grants NS19608, MH00058, and NS09076.)

METHAMPHETAMINE INCREASES HYDROXYL RADICALS IN RAT STRIATUM: ROLE OF DOPAMINE. A. Giovani, T.G. Hastings, L.P. Liang, and M.J. Zigmund. Department of Cellular and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

The role of dopamine (DA) in the induction of dopaminergic neurotoxicity resulting from methamphetamine (METH) treatment supports the hypothesis that DA may be involved in the etiology of Parkinson's disease. DA can autooxidize to form a variety of potentially toxic compounds, including free radicals such as the hydroxyl radical (OH). To assess the role of free radicals in the toxic effects of METH, we used salicylate to trap OH- in vivo. We then used HPLC with electrochemical detection to measure the striatal content of 2,3-dihydroxybenzoic acid (2,3-DHBA), stable adducts resulting from OH- attack upon salicylate (see Liang et al., Soc. Neurosci. Abst., 1992). Adult male rats (325-375 g) were given a neurotoxic regimen of METH (5 mg/kg, s.c., 4x, at 2 hr intervals) and neurotoxicity was assessed by examining striatal DA content for long-term depletion. Some rats received salicylate 1 hr after the last dose of METH and the animals sacrificed 2 hr later. Under these conditions, METH caused a significant increase in the level of 2,5-DHBA in striatum, from 107 ± 13 to 237 ± 34 pmol/mg tissue (mean ± SEM, n=8, 9, respectively). This increase, as well as the decrements in striatal DA, was blocked by pretreatment with a methamphetamine antagonist (5 mg/kg, i.p.), an inhibitor of catecholamine biosynthesis. These results indicate that: 1) the formation of free radicals in striatum increases in response to neurotoxic doses of METH; and 2) DA plays an essential role in this METH-induced increase in free radicals. These findings also suggest that under other conditions of increased availability, endogenous DA may play a role in neurodegeneration through the formation of free radicals. (Supported in part by NS19608, MH18273, and MH00058.)


We recently proposed a new mechanism for MPP+ induced oxidative stress, involving a two electron transfer, called hydride transfer. This process is mediated by a number of enzymes, especially flavin enzymes. Hydride transfer to MPP+ results in the formation of 1-methyl-4-phenyl-1,4-dihydriodipridine (DHP) which is quickly oxidized by O2 in one electron steps. The first product is MPP+ which can reduce oxygen to form O2- or cleave hydrogen peroxide to form H2O2 and regenerate MPP+. We now report the ratio of O2- to H2O2 and OH- during incubations of MPP+ or MPDPP+ with enzymes including monoamine oxidase, lipomide dehydrogenase, aldehyde dehydrogenase and xanthine oxidase. These experiments in addition to oxygen uptake experiments are demonstrating the possibility of MPP+ to reduce cyclo and form radicals that could be damaging to cells. Most of the enzymes examined are found in mitochondria, and perhaps the cytosol, which may indicate the importance of oxygen radical formation by MPP+ in mitochondria. This redox cycling can complement the formation of O2- during inhibition of NADH dehydrogenase by MPP+. Mitochondrial and cytosolic generation of oxygen radicals are critical components of the toxicity induced by MPP+.


In Parkinson's disease (PD), iron content and lipid peroxidation is increased within the substantia nigra (SN). We recently reported that iron infusions into the rat SN induce localized neurodegeneration as well as dose dependent reductions in striatal dopaminergic markers (Snypers et al., Exp. Neurol. 129, 364-373, 1997). In the present study, we measured the changes in the MDA levels and striatal and nigral levels of the dopamine (DA) metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) in the SN and striatum in response to eight daily infusions of either 1 or 5 mg iron/kg.Following iron infusions into the SN we observed significant increases in the MDA levels, is acutely increased following iron infusion into the SN. In response to one MCDT, we observed significant increases in the MDA levels, is acutely increased following iron infusion into the SN. In response to one MCDT, we observed significant increases in the MDA levels, is acutely increased following iron infusion into the SN. In response to one MCDT, we observed significant increases in the MDA levels, is acutely increased following iron infusion into the SN.

Neurodevelopmental disorders (NDDs) such as learning disabilities, articulation disturbances, attention deficit disorder, cerebral palsy, and mental retardation may sometimes be triggered by events that occur during gestation. One important intraterine factor may be endocrinological: it has been suggested that imbalances of sex hormones are associated with neurodevelopmental disorder. Since hormone levels are known to vary seasonally, the relationship between hormonal variation in utero and NDD was indirectly assessed by measuring the incidence of NDD for children conceived during different seasons (defined in terms of duration of daylight). This was examined in a subsample of The National Collaborative Perinatal Project (N = 21,833). There were seasonal variations in the rate of NDDs with the highest prevalence for winter/fall conceptions as compared to spring or summer. These findings were robust since they remained unchanged when considering seasonal variations in other risk factors such as maternal weight gain, maternal anemia, and maternal infections.

This work was supported by NIMH Grant # R03 MH47040 to J.L.

604.3 SPECT EVIDENCE OF DELAYED FRONTAL CORTEX MATURATION IN CHILDHOOD AUTISM M. Zilbovicius*, B. Gareau, V. Samson, Ph. Remy, B. Bruck, C. Barthélémy, A. Syrotu, G. Letoile. SHFJ, CEA, Orsay and INSERM U316, Tours, France.

Neurobiological causes of childhood autism, a severe developmental disorder, are still unknown. Clinical and cognitive data have suggested that the maturation of the cerebral cortex may be delayed in this disorder, particularly involving the frontal lobe. Maturation of the frontal cortex can currently be assessed by measures of regional metabolism (Chugani and Phelps, 1986) or cerebral blood flow (Territorio et al., 1988). We used SPECT to compare the regional distribution of cerebral blood flow (rCBF) in young children with prenatal G 14-7 8 months) and in five age-matched non-autistic children (33±6 months). All studies were performed under sedation with the Xenon 133 iv injection method. The normalized frontal rCBF was markedly higher in the autistic group (0.86±0.07) than in controls (0.96±0.08, p<0.0001). The normalized posterior cortical rCBF was slightly higher in autistic than in control children (1.10±0.25 vs. 1.07±0.25, p<0.04). The mean cerebral blood flow was similar in both groups (autistic: 73.2±10.1, control: 72.4±8.5 ml/min/100g; control: 72.4±8.5 ml/min/100g, ns). We believe that the marked frontal hypoperfusion found in these young autistic children (2-4 years) may indicate a delayed frontal lobe maturation rather than a frontal hyperactivity since 1) such frontal hyperperfusion is found in normal but younger children (1-2 years old), and 2) frontal hyperperfusion is normal in older (5-11 years old) autistic children (Zilbovicius et al., 1992). Supported by INSERM network 489001 and Fondation Fyssen.


Spontaneously hypertensive (SHR), Sprague-Dawley (SD) rats neonatally deprived of frontal corticostriatal dopamine by 6-hydroxydopamine injection (600, 100 µg/ml) or made micromephic by prenatal methylazoxymethanol (MAM; 25mg/kg) injection to pregnant dams, and post-weaning social isolation (SI) and prior histamine injections in SHR were determined. Locomotor activity and rearing frequencies during FT session 1 were determined. The normalized frontal rCBF was markedly higher in the autistic group (0.86±0.07) than in controls (0.96±0.08, p<0.0001). The normalized posterior cortical rCBF was slightly higher in autistic than in control children (1.10±0.25 vs. 1.07±0.25, p<0.04). The mean cerebral blood flow was similar in both groups (autistic: 73.2±10.1, control: 72.4±8.5 ml/min/100g, ns). We believe that the marked frontal hypoperfusion found in these young autistic children (2-4 years) may indicate a delayed frontal lobe maturation rather than a frontal hyperactivity since 1) such frontal hyperperfusion is found in normal but younger children (1-2 years old), and 2) frontal hypoperfusion is normal in older (5-11 years old) autistic children (Zilbovicius et al., 1992). Supported by NICHHD grant # PO HD26927.

604.5 EFFECTS OF METHYLAZOXYMETHANOL (MAM) TREATMENT ON GESTATIONAL DAY 14 (GD14) ON NEUROCHEMISTRY, REGIONAL BRAIN WEIGHT, AND BEHAVIOR. S. A. Ferguson, B. R. Holton, M. G. Paile and J. F. Bowyer. Divisions of Neurotoxicology and Reproductive & Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Sprague-Dawley rats were injected sc with either MAM (20 mg/kg) or saline on GD14 (plug date=GD 0). Beginning prior to weaning and continuing through adulthood, offspring were assessed on a variety of behavioral tests, including activity measures. Regional brain weights were obtained and DA and 5-HT concentrations in certain brain regions were measured. Contrary to what is typically reported, MAM-treated rats exhibited either hypopreactivity or normal activity levels. No consistent indications of hyperreactivity were noted. Frontal cortex, hippocampus, and CN of MAM-treated rats were most reduced in weight (cortex weight was 50% of controls while brainstem, olfactory bulbs, thalamus, cerebellum, and body weight were relatively unaffected). MAM treatment increased 5-HT concentrations in CN two-fold, with little effect on DA concentrations. Regional brain weight reductions and increased CN 5-HT concentrations in MAM-treated rats were consistent with previous findings; however, neither activity nor DA levels were consistent with literature reports. The possibility that these data may reflect a strain difference cannot be excluded.

604.6 AN INBRED EPILEPSY-PRONE SUBSTRAIN OF BALB/c MICE SHOWS AN ABNORMAL PROJECTION TO BASAL FOREBRAIN. S. Dolina*, C. Morin, C. P. Rojan and R. T. Robertson. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Epilepsy-prone (EP) and epilepsy-resistant (ER) substrains of mice derived from the BALB/c strain have been developed through 19 generations of close breeding and subsequent inbreeding. The EP strain shows the acallosal phenotype in about 15% of the population. In this study, we determined the presence or absence of corpus callosum (CC) in EP and ER mice. Data collected from offspring (postnatal age 13-14 days) and adult mice. Animals from the 4th and 5th inbred generations were used. Postnatal saline was injected to Niais strain and D1 for the tracing of cortical afferents. Er animals had normal corpus callosum (101 cases). Twenty of 25 EP animals were acallosal and the remaining 6 EP animals displayed a corpus callosum markedly reduced in size. Placement of small crystals of D1 in motor cortex of ER animals revealed the presence of a normal corpus callosum, with anterograde labeling of terminals and retrograde labeling of pyramidal neurons in the contralateral cortex. D1 labeled axons extended toward the midline, but turned ventrally to course through the basal forebrain. These fibers appeared to terminate in the basal forebrain in the region of the nucleus of the diagonal band. EP animals with a normal corpus callosum showed some labeled fibers crossing the midline to terminate in the contralateral cortex, but many fibers also coursed ventrally to the basal forebrain. These results show that aberrant cortical projections to the basal forebrain occur in mice that lack a corpus callosum.
4.4 DISTRIBUTION AND MORPHOLOGY OF THE CORTICOSPINAL TRACT NEURONS IN PRENATALY X-RAY IRRADIATED RATS. RETROGRADE TRACING AND INTRACELLULAR LUCIFER YELLOW STAINING STUDIES.


X-ray irradiation to fetus is a potent teratogenic procedure, and such it is the prime cause of mental retardation and behavioral abnormality. In order to clearify the effect of X-ray irradiation on development of the corticospinal (CS) tract neurons, fetal rats were irradiated with X-ray (1.5 Gy, 140 kV, 4 mA) at pregnant day 12. At the day of birth the ewes were sacrificed either by decapitation or by transcardial perfusion with saline followed by 4% paraformaldehyde. The brains were post-fixed for 28-96 hours, sectioned by transcardial perfusion with saline followed by 30% buffered sucrose for 24 hours, after which they were frozen and cut coronally at 30 μm. Immunohistochemistry was performed on adjacent sections.

Neurons destined to form particular layers (Rakic, '74-'88). Macaque fetuses were used to examine the effect of X-ray irradiation on the morphological and cellular components of the cortex. X-irradiation was performed between P60 and 2 yrs. The brains were cut at 35 μm from celloidin blocks and immunostained with specific antibodies for various neuronal markers and nuclei. The results of this study indicated that X-ray irradiation results in a significant reduction of the number of CS neurons, particularly in layer V, with a preferential reduction in the intermediate layers IV and V. The effects of X-ray irradiation on the corticospinal tract neurons were also investigated using cell counting methods (Williams & Rakic, '88). The effects of doses and schedules of X-irradiation were then assessed and compared.

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Pyramidal Cells in the Sensory-motor Cortex of the H-Tx rat with infantile hydrocephalus. H.C. Jones* and H.D. Harkay. Dept. of Pharmacology, Univ. of Florida, Gainesville, FL 32610 and # Dept. of Physiology, King's College, London W 8 TAH, UK.

The H-Tx rat has congenital hydrocephalus caused by obstruction of the aqueduct at 18-days gestation. The lateral ventricles expand rapidly after birth and the cortical grey matter becomes severely attenuated, particularly in the posterior cortex where there is a disruption of the laminar structure in the deeper layers. Layer V pyramidal cells have been studied at 21 days after birth by quantitative analysis of Golgi-stained sections particularly in the posterior cortex where there is a significant reduction in the number of dendrite rings by quantitative analysis of Golgi-stained sections. This was confirmed by concentric circle analysis which showed a reduction in the number of dendrite-ring intersections with increasing distance from the soma. The angle of the apical dendrite to the pial surface was significantly reduced by 40 - 60% in total length and also in vertical and horizontal extent when compared to control rats. This was confirmed by concentric circle analysis which showed a reduction in the number of dendrite-ring intersections with increasing distance from the soma. The angle of the apical dendrite to the pial surface was significantly reduced by 40 - 60% in total length and also in vertical and horizontal extent when compared to control rats. The basal dendrites, although variable, were not significantly altered in cells from hydrocephali in either vertical or horizontal extent. The results are consistent with ventricular expansion resulting in compression of the deep layers of the cortex and also with tangential forces causing disorientation of pyramidal cells.

Effects of Hydrocephalus and Decompression on Retinal Cell Development. E.C. Williamson, H.E. Pearson, T.J. Shickley, and J.P. McAllister II. Temple University School of Medicine, Philadelphia, PA 19140.

Even after surgical decompression, infantile hydrocephalus often results in permanent neurological symptoms, including visual deficits. However, little is known about the cellular changes that may be responsible for these problems. The present study was designed to analyze the retinae of normal, severely hydrocephalic, and decompressed kittens to determine the density and size of retinal ganglion cells. Hydrocephalus was induced in 10 day old kittens by intra-cisternal injection of kaolin. Kittens were allowed to survive from 7-28 days and then sacrificed and perfused with mixed aldehydes. Animals that received ventriculoperitoneal shunts were shunted 10-15 days after the induction of hydrocephalus and then sacrificed 10-14 days after shunt placement. Retinae were flat-mounted onto glass slides and stained with cresyl violet. A 750 x 750 micron sample area was chosen 1 cm along the horizontal meridian in nasal retinas, and cells were drawn using a drawing tube under 40X magnification. Cell density and cell area were determined using the Bioquant image analysis system. Total cell density was significantly increased in severely hydrocephalic animals but returned to within normal levels following decompression. On the basis of cell size, however, the glial population was significantly increased and there was a significant loss of ganglion cells in both the hydrocephalic and the shunted groups. Based on the results, we conclude that gliosis occurs as a result of cell death in the retina following hydrocephalus, and decompression is unable to reverse these effects. Supported by NS25106 and HD21527.


Expression of alternatively spliced forms of mRNA, which encodes neurofibromin, has been identified by RNAse protection assays. The presence of the alternatively spliced form is associated with a range of neurological and developmental disorders including the human disorder neurofibromatosis type 1 (NF-1), the chicken insert, as well as the actual site of insertion, is identical to that for human neurofibromin. RNAse protection and RNA-PCR analyses were used to assess levels of expression of the alternatively spliced neurofibromin mRNA in the chicken embryo. Most tissues express predominantly the longer form early in development and type I later. We are currently performing in situ hybridization to determine the tissue localization of the two forms of mRNA. Supported by the Oregon MRF.
**SYMPOSIUM: GABA$_B$ RECEPTORS AND THEIR ROLE IN NEUROTRANSMISSION, NEUROMODULATION AND NEUROPATHOLOGY.**


GABA$_B$ receptors are found in the mammalian brain. The symposium will attempt to cover most aspects of these phenomena. N.G. Bowery will provide an overview of past and present concepts of the characteristics of GABA$_B$ receptors and will consider their role in diseased states. R.A. Nicoll will discuss the significance of GABA$_B$ receptors in the hippocampus, the brain region from which much of our knowledge of their function derives. H. Bittiger will introduce new GABA$_B$ receptor antagonists and will provide substantial evidence for a role for GABA$_B$ receptors in the genesis of absence seizures. Finally, K. Kuriyama will provide information about recent progress in the purification and structural analysis of the GABA$_B$ receptor.

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**APOLIPOPROTEIN E IN GLIAL CELLS OF THE RAT OLFACTOR Y BULB: POSTNATAL DEVELOPMENT AND RESPONSE TO DEAFFERENTATION.**

G.E. Handelman* and M.L. Russell. Dept. of Pharmacology, Univ. of Utah, Salt Lake City, UT 84112.

Apolipoprotein (apo)E, a lipid transport protein, has been proposed to play a role in nerve regeneration. We therefore studied apo-E distribution, development, and response to injury in the olfactory system, the only region of the mammalian CNS in which true nerve regeneration is known to occur. Using single and double label immunocytochemistry, we found apo-E immunoreactivity (IR) in astrocytes in all layers of the adult olfactory bulb. Apo-E-IR was extensively colocalized with GFAP. In the olfactory nerve, apo-E was present in cell bodies and occasional fibers possibly of those of the ensheathing cells, a type of astroglia. In rat pups, apo-E was present in the olfactory nerve as early as 2 days postnatal, although very little adult pattern of apo-E IR was reached after 3 months of age. The distribution of the astrocytes was unchanged, but they contained more GFAP- and apo-E-IR. The morphological appearance of reactive astrocytes is similar. Deafferentation of the bulb in young adults, induced by zinc sulfate injection of the nasal epithelium, also increased apo-E and GFAP-IR in all layers. The effects were most pronounced in the glomerular layer, the site of the olfactory nerve terminals, and within the olfactory nerve. These results indicate that apo-E is synthesized by glia of the olfactory nerve and bulb, and that its synthesis is increased in response to aging and nerve injury. The increased synthesis may be elicited by a factor associated with axonal degeneration.

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**GLIAL AND OTHER NON-NEURONAL CELLS VII**

**EXPRESSI ON OF 84 INTEGRIN IN RAT PERIPHERAL NERVE IS DEVELOPMENTALLY REGULATED AND ANXIONX MODULATED DURING REGENERATION.**

M.L. Feltri*, S. Scherle, J. Wrabetz, H. Vogelback, L. Barsoum, P. Shy, P. Kamholz, S. Raffaele Hospital, Milano, Italy; University of Pennsylvania, Children's Hospital of Philadelphia and Thomas Jefferson University, Philadelphia, PA, 19104.

Integrins are a family of receptors implicated in cell adhesion and cell signaling whose ligands include extracellular matrix proteins. Myelinating Schwann cells (SC) require various basement membrane components to initiate myelination. Since expression of the 84 integrin subunit has been shown to be restricted to epithelial cells and SC, we chose to study expression of 84 integrin in postnatal sciatic nerve development and after crush injury. Using 84 integrin cDNA from a rat peripheral nerve library, Northern blot analysis demonstrated that 84 integrin mRNA is present at low levels postnatal day 1 and steadily increases from day 15 to at least day 90. After a crush injury, which results in Wallerian degeneration followed by prompt axonal regeneration, 84 integrin mRNA expression falls markedly by 4 days post-traumatic reepithelialization and peaks by 8 days and peaks by 12 days, paralleling the time course of axonal regeneration. Furthermore, 84 integrin mRNA expression is induced in forskolin-stimulated SC culture, an in vitro model of SC axonal signaling. We conclude that the sciatic nerve 84 integrin expression is axonally modulated, suggesting that 84 integrin may function in axon-SC interactions during myelination.
The projection of the zinc-containing axons of hypothalamic astrocytes suggests that these cells may play a role in the expression of its own gene, and b) hypothalamic astrocytes express E2 receptors which mediate a facilitatory effect of E2 on TGF-α. Protection assays revealed the presence of E2 receptor mRNA in cultured astrocytes of sector CA4 and CA3 might be a candidate for a specific neuron-glial interaction which results in the outgrowth of the mossy fibers to sector CA3 and CA4.

**608.6 REGULATION OF A NOVEL CYCLOOXYGENASE (PROSTAGLANDIN G/H SYNTHETASE) GENE BY DEXAMETHASONE AND CALCIUM IONOPHORE IN ASTROCYTES.** D. L. Coleman and P. D. Coleman, Departments of Neurology and of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

In astrocytes, dexamethasone (1 μM, 4 h) reduces the level of E1 mRNA by 2.3-fold without altering the 2.8 kb mRNA level. This inhibition occurs in the presence of cycloheximide which alone increases 4.1 kb mRNA levels by nearly 10-fold without super-induction of the 2.8 kb mRNA within 3 h. Preliminary results suggest that the induction of 4.1 kb mRNA by ionophore is transient. These observations are consistent with our previous studies in mouse fibroblasts and human monocytes and suggest that the 4.1 kb mRNA encodes the predominant regulated cyclooxygenase in astrocytes. Supported by [LEAD award](#).

**608.7 REGULATION OF TRANSFORMING GROWTH FACTOR ALPHA (TGF-α) mRNA EXPRESSION IN RAT HYPOTHALAMIC ASTROCYTES BY TGF-β AND ESTRADIOL (E2). Y. Ma, M. Mochib-Sieber, D. H. and S. Glade, Div. Neurosci., Oregon Regional Primate Research Center, Beaverton, OR 97006.** TGF-α, a mitogenic polypeptide that acts via activation of epidermal growth factor receptors (EGFR), has been implicated in the neuropathological mechanism by which hypothalamic lesions induce sexual precocity and the neuroendocrine process that underlies the initiation of normal female puberty. TGF-α appears to exert these effects via stimulation of tissue hormones releasing hormone (LHRH) secretion. Since both TGF-α and EGFR expression in the hypothalamus are predominantly astroglial, and LHRH neurons are devoid of EGFR, we have postulated that TGF-α facilitates LH-RH release indirectly via an autocrine/paracrine-mediated activation of glial function. To begin examining this hypothesis, hypothalamic astrocytes cultured in defined medium were exposed to either TGF-α (50 ng/ml) or a phorbol ester (TPA, 10 ng/ml) for various lengths of time. The effect of these agents on TGF-α mRNA was assessed by reverse transcriptase assay. TGF-α mRNA was readily detected in untreated astrocytes; both TGF-α and TPA increased TGF-α mRNA levels to maximal values (3-4-fold increase) by 8h. Additional experiments were performed to determine if glial TGF-α mRNA levels are regulated by E2. E2 protection assay revealed that E2 decreases TGF-α mRNA levels in cultured hypothalamic astrocytes. E2-17β (1 nM), but not E2+T, induced a twofold increase in TGF-α mRNA within 8h of treatment. These results suggest that: a) TGF-α can act in an autocrine fashion on hypothalamic astrocytes to enhance expression of its own gene, and b) hypothalamic astrocytes express E2 receptors which mediate a facilitatory effect of E2 on TGF-α gene expression.


Studies conducted by this laboratory have established that in culture, local substantia nigra (SN) support cells contribute to the survival of the dopaminergic neurons selectively lost in Parkinson's disease. Fractionation of embryonic SN glia into enriched oligodendrocyte, type I or type II astrocyte subpopulations has modified the type I astrocytes as the source of the soluble neurotrophic factor(s) for SN dopaminergic neurons. These well-characterized SN glial populations have been utilized in subtractive cloning methodologies to identify genes specifically expressed by type I astrocytes. The support cell fractionation generated >70% homogeneous glial subpopulations, from which total RNA was harvested. The liming amount of RNA obtained necessitated development of a subtractive cloning technique using the polymerase chain reaction (PCR). In the library described, sequences in common between type I and II astrocytes were removed by stringent hybridization and hydroxyapatite (HAP) chromatography. Ten cDNA clones have been isolated from the type I cells which are not expressed or expressed at relatively low levels in type II astrocytes. Nucleotide sequence analysis of the type I specific cDNAs have indicated the isolation of novel open reading frames with no significant homology to existing Genebank or EMBL sequences. In addition, two cDNAs contain novel sequences with the exception of their 3' untranslated regions which contain a 50 base pair stretch identified as a rat repetitive element (SINE). Further analysis of these type I astrocyte specific cDNA clones will give insight into the molecular basis of the type I astrocyte neurotrophic effect on SN dopaminergic neurons. In addition, subtractive cloning approaches reported here may be used to analyze other glial populations from discrete brain regions.
608.11

GLIAL AND OTHER NON-NEURONAL CELLS VI

RECEPTORS COUPLED TO PHOSPHOLIPASE C. G.F. Graminski, ANALYZE LIGAND EFFECTS ON G-PROTEIN LINKED phospholipase C second-messenger pathway. The assay is based on
Haven, CT 06510.

C.K. Jayawickreme, A. Roby-Shemkowitz and M.R. Lerner*, Depts. of Cell
are potent stimulators of pigment dispersion in the microglial reactions in several experimental neuropathologies. These
activation. To test this hypothesis, pigment cells were transiently agonist induced darkening of pigment cells. A distinct activation of perivascular microglial cells and an involvement of perineuronal microglial cells in the deafferentation (synaptic stripping) of afferent synaptic terminals from the surface of
resting microglial cells form a network of regularly spaced, ramified cells throughout the CNS. Studies on their reactions after facial nerve axotomy have suggested that they function as an intrinsic immune defense system within the CNS. To further examine this hypothesis in vivo, we have studied the microglial reactions in several experimental neuropathologies. These include retrograde reactions of motoneurons, anterograde reactions, combined anterograde and retrograde reactions and recently neurotoxin models. In addition, we have studied glial ischemia and T cell-mediated autoimmunity of the central and peripheral nervous system. From these results it has become evident that microglial cells (but not astrocytes) rapidly proliferate and increase the expression of several immunologically relevant molecules, such as MHC class I and II antigens. Their activation is further observed rapidly if the site of the microglial activation is damaged. At the ultrastructural level, two phenomena among several other things were observed: a distinct activation of perivascular microglial cells and an involvement of perineuronal microglial cells in the deafferentation of neurons suffering e.g. from nerve trauma. In summary, microglial cells thus appear to be the main immune effector cell population of the CNS.

608.13

WITHDRAWN

608.14


American trypanosomiasis [Chagas' disease caused by Trypanosoma cruzi (T.c.)] and toxoplasmosis [caused by Toxoplasma gondii (T.g.)] are two protozoan diseases where nervous system involvement is increasingly widespread in immunosuppressed individuals. Infection of myocardial cells with T.c. results in asynchronous conduction and gap junction loss [Circ. Res (92)70:733]. To examine whether similar changes occurred in cells of brain, pure cultures of leptomeningial cells or astrocytes were infected with T.c. or T.g. and junctional communication and connexin abundance and intercellular distribution were examined. For either type of cell infected with either parasite, intercellular coupling (injected Lucifer Yellow) offers great utility and speed for analyzing the pharmacological potencies of agonists and antagonists linked to phospholipase C.

609.1


A melanophore based bioassay system has been developed for rapidly evaluating ligand effects on recombinant receptors coupled to the phospholipase C second-messenger pathway. The assay is based on agonist induced darkening of Xenopus laevis melanophores resulting from pigment dispersion. Both TPA and the Ca2+ ionophore A23187 are potent stimulators of pigment dispersion in Xenopus melanophores, suggesting that pigment dispersion is mediated via phospholipase C activation. To test this hypothesis, pigment cells were transiently transfected with the phospholipase C activating mutant GTPase receptor. Treatment with bombesin and other agonists induced pigment dispersion in cells expressing the bombesin receptor but not in wild type cells. The responses were dose dependent. EC50 values for bombesin (0.12 nM) and other agonists such as loriotin (0.15 nM) and neuropeptide B (1.3 nM) were comparable to the literature values. The bombesin receptor antagonist, [D-Phe2]bombesin 13 methyl ester, inhibited pigment dispersion with an IC50 value of 15 nM. Bombesin stimulation provoked a substantial increase of 1,4,5-IP3 production over basal levels while no rise in AMP was observed. This bioassay system potentially offers great utility and speed for analyzing the pharmacological potencies of agonists and antagonists associated with receptors linked to phospholipase C.

609.2


We have created a versatile and sensitive visual assay for the expression of receptors linked to G-proteins. Transient transfection of Xenopus melanophores with plasmids containing cDNAs for either the beta adrenergic (Gβ), the substance P (phospholipase C), or the D dopamine receptor (Gβ) at dilutions of 1 plasmid in 10,000 were detectable. Computerized subtraction of video images taken pre- and poststimulation allowed the detection of individual responses in fields of 10,000 cells. Activation of phospholipase C and adenylyl cyclase caused dispersion of melanosomes (organelles containing melanin) while inhibition of adenylyl cyclase caused melanosome aggregation. Responding cells appear to require only a single copy of a plasmid containing a receptor cDNA. This assay is capable of conveniently screening several hundred thousand cDNA clones per day.

Atrial natriuretic peptides (ANP) are synthesized and localized in different brain regions. Neurotensin peptide binds to specific receptors and generate cGMP as the second messenger. When ANP receptors were stimulated with 5 and 10 nM ANP (99-126) a marked increase in activity of protein kinase C (PKC) in cytosolic fraction of the diaphragm occurred. The membrane PKC level was not altered significantly with treatments up to 5 nM ANP, whereas the 10 nM ANP produced a 4-fold increase.

The increase in cytosolic PKC was 80% above control with 5 nM ANP treatment of the diaphragm slices and maximal induction was observed following 30 min treatment. Incubation of ANP (5 µM) with actinomycin D (10 µg/ml) or cycloheximide (20 µg/ml) blocked the induction of PKC to 90% and 84%, respectively. This indicated the increase in cytosolic PKC resulted from de novo protein synthesis. Pertussis toxin (5 ng/ml) attenuated the induction of PKC by 30% suggesting Gi proteins are involved in the ANP mediated PKC induction. cGMP levels in 0.001 and 0.01 µM ANP treated diaphragm slices increased 5.4 fold and 3.8 fold respectively compared to controls. Incubation of slices with 0.1, 0.5, and 5 µM ANP showed a significant increase in cGMP levels. These results suggest increases in cytosolic PKC levels alter the guanylate cyclase-coupled receptor function.


SR 48692 was reported to be a potent non-peptide antagonist of Neurotensin (NT) receptors and effects in both guinea-pig and murine species. (D. Gulli et al., P. Klidias et al., this volume). In order to test whether this compound also had any activity in humans, homogenates from newborn or adult human cortices were prepared. Binding studies demonstrated that SR 48692 competitively inhibited specific binding of 125I-SR 48692 with an IC50 of around 12 nM. Autoradiographic data obtained at the level of the substantia nigra and nucleus paraganglia of normal human tissue sections demonstrated a dose-dependent inhibition of 125I-SR 48692 labeling with increasing concentrations of SR 48692. Similar data were obtained on 125I-SR 48692 binding in primary cultures of human mesencephalic neurons. In a cell line, derived from a colon carcinoma (HT29), SR 48692 competitively antagonized NT-induced intracellular calcium mobilization obtained by means of flow cytometry using indo-1 fluorescence. The observed pA2 values (mean 8.1 nM) were consistent with results obtained in binding studies (K 20 nM). Moreover, in this model, SR 48692 was devoid of any intrinsic agonist activity. In conclusion, this potent non-peptide antagonist of NT receptors may help to understand the putative pathological roles of NT.


Galanin,29 (GAL) is a neuropeptide with several biological activities in the brain, such as behavior and interaction with 5-HT1A and acetylcholine receptors. In most cases N-terminal fragments such as GAL1-15, but not C-terminal fragments, have been shown to have biological activities of the whole peptide.

We have analyzed the distribution of 125I-GAL1-15 (specific activity 2000 Ci/mmole) binding sites in the rat brain using quantitative receptor autoradiography. The distribution was different from 125I-GAL1-29 binding sites, but also with several overlapping regions. Most notably, 125I-GAL1-15 binding sites were present in the dorsal hippocampus, the neostriatum and the neocortex, areas almost lacking 125I-GAL1-29 binding sites in the rat brain. The 125I-GAL1-15 binding was more readily displaced with GAL1-29 than with GAL1-29 indicating the presence of a binding site with higher affinity for the fragment. The fragment binding site was saturable with a Kd of 0.63 ± 0.02 nM and a Bmax of 15.3 ± 0.1 fmol/mg protein in the dorsal hippocampus and a Kd of 0.44 ± 0.09 nM and a Bmax of 15.8 ± 0.5 fmol/mg protein in the ventral limbic cortex. Non-specific binding was determined as the binding in the presence of 10 µM of GAL-15. The specific binding was approximately 80% at Bmax. The present study gives evidence for the existence of specific binding sites for 125I-GAL1-15 fragment different from the GAL1-29 receptor which may represent a unique GAL fragment receptor.
609.3
REGIONAL DISTRIBUTION OF CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS IN THE TREE SHREW BRAIN. E. Fuchs*, M. Weinrich and G. Flügge. German Primate Center, Goettingen, FRG.

CRF is the predominant messenger in the control of the activity of the pituitary-adrenal axis and is therefore ultimately responsible for triggering the endocrine responses to stress. In the present project, we investigated the influences of chronic stress on central nervous CRF receptors. In our studies we used tree shrews (Tupaia belangeri), a species which provides a useful model for studying the outcome of psychosocial stress on the central nervous system. In the first part of the study we localized and quantified the receptors in the brains of control animals by autoradiography with [125I]-labeled ovine CRF. It revealed the presence of receptors with a high and a low affinity component. High densities of specific CRF receptors were localized in the tractus olfactorius, the dentate gyrus, the outer layers of cortex, the superior colliculus, the cerebellum, the vernal nucleus, and the pituitary (anterior part, intermediate zone). Moderate concentrations were present in the amygdala and the lateral septum. Lower densities were found in the caudate nucleus and the locus coeruleus. These results indicate species differences with respect to distribution and densities of CRF receptors. In the second part of the study we are currently investigating whether chronic psychosocial stress induces changes of CRF receptor affinities and/or densities of these receptors.

610.1
"THERAPEUTIC WINDOW" FOR NMDA ANTAGONIST PROTECTION AGAINST FOCAL CEREBRAL ISCHEMIA MAY BE NARROW. G.K. Steinberg*, N.M. Pennigan, G.H. Sun. Dept. of Neurosurgery, Stanford University Medical Center, Stanford, CA 94305.

While NMDA antagonists have been shown to protect against focal cerebral ischemia, it is not clear how long therapy can be delayed and still achieve neuroprotection. Under halothane anesthesia, 55 rabbits underwent 2 hour occlusion of the left internal carotid, anterior cerebral and middle cerebral arteries followed by 4 hours of reperfusion. Rabbits were treated with either i.v. normal saline (n=10) or the NMDA antagonist dextrophan (DX) as follows (n=5/group): 1 hour delay: 5, 10 or 15 mg/kg/hr; 2 hour delay: 7.5, 12.5, 17.5 mg/kg/hr; 4 hour delay: 10, 15 or 20 mg/kg/hr. Injury was assessed using magnetic resonance imaging for ischemic edema (IND) and either WGA-HRP or FG as retrograde tracers. Rabbits were treated with either i.v. normal saline control: 45 ± 7% IND; 47 ± 3% edema, p<0.05. Protection against striatal IND was found only with 2 hour DX delay (17.5 mg/kg group: 37 ± 17%, 12.5 mg/kg group: 50 ± 15% vs. control: 83 ± 6%). DX treatment delayed 4 hours worsened the ischemic edema (15 mg/kg group: 70 ± 5% IND, 29 ± 8% edema). Injury was assessed using magnetic resonance imaging for ischemic edema (IND) and either WGA-HRP or FG as retrograde tracers. Rabbits were treated with either i.v. normal saline control: 45 ± 7% IND; 47 ± 3% edema, p<0.05. Protection against striatal IND was found only with 2 hour DX delay (17.5 mg/kg group: 37 ± 17%, 12.5 mg/kg group: 50 ± 15% vs. control: 83 ± 6%). DX treatment delayed 4 hours worsened the ischemic edema (15 mg/kg group: 70 ± 5% IND, 29 ± 8% edema). Injury was assessed using magnetic resonance imaging for ischemic edema (IND) and either WGA-HRP or FG as retrograde tracers. Rabbits were treated with either i.v. normal saline control: 45 ± 7% IND; 47 ± 3% edema, p<0.05. Protection against striatal IND was found only with 2 hour DX delay (17.5 mg/kg group: 37 ± 17%, 12.5 mg/kg group: 50 ± 15% vs. control: 83 ± 6%). DX treatment delayed 4 hours worsened the ischemic edema (15 mg/kg group: 70 ± 5% IND, 29 ± 8% edema).

610.2

The effect of the NMDA receptor blocker MK-801 (dizocilpine maleate) was evaluated both early (3 days) and late (28 days) after middle cerebral artery (MCA) occlusion. Injury was estimated from 8 histological sections of defined levels of the brain. A 40% (p < 0.05) reduction of infarct size was found in MK-801 treated rats studied after 3 days. This effect was not found 28 days after MCA occlusion, where no difference in infarct size or final tissue loss (infarct volume + ipsilateral hemisphere atrophy) was seen between the MK-801 and placebo-treated rats. There was no significant effect of MK-801 on the ipsilateral hemisphere volume (reflecting edema) at 3 days.

Thus, MK-801 seemed to have a transient attenuating effect on the ischemic process itself with no apparent influence on the edema component. This study underlines the importance of including a late end point when evaluating the efficacy of neuroprotective stroke therapy. It remains to be shown whether or not multiple dose treatment with NMDA receptor blockers attenuate the final neuropathologic outcome after experimental stroke.


610.3

In culture the excitatory and stimulatory effect of L-glutamate (Glu) on neurons results in neuronal death through a mechanism involving the persistent translocation of PKC and the destabilization of [Ca2+]i homeostasis. In contrast, intermittent Glu receptor use elicits the coordinated expression of immediate early genes (IEG) acting as transcriptional activators of immediate early gene (IEG) expression. These results indicate species differences with respect to distribution and densities of CRF receptors. In the second part of the study we are currently investigating whether chronic psychosocial stress induces changes of CRF receptor affinities and/or densities of these receptors.

610.4
REVERSIBLE INACTIVATION OF CALCIUM/CALMOLUIN KINASE II INDUCED BY SPINAL CORD ISCHEMIA. D.A. Shackelford, R.Y. Yeh and J.A. Zivin*. Dept. of Neurosciences, Univ. of Calif. at San Diego, La Jolla, CA 92039-0624.

It has been proposed that ischemia-induced neuronal damage is caused by an accumulation of extracellular glutamate leading to an influx of extracellular Ca2+ through membrane channels disrupting Ca2+ homeostasis. The activity of Ca2+/calmodulin-dependent protein kinase II, which is involved in the regulation of neurotransmitter release, neuronal plasticity, and apoptosis, has been shown to decrease after ischemia in several models. In the present study, the kinetics of inactivation and fate of the protein in the rabbit spinal cord ischemia model were analyzed. The Ca2+/calmodulin-dependent activity of CaM kinase II, measured by incorporation of phosphate into exogenous peptide substrates, was decreased rapidly such that after 10 min of ischemia, which precedes irreversible paraplegia, 65% and 40% of the activity was lost from the cytosolic and particulate fractions, respectively. Therefore, analyses of CaM kinase II activity in the particulate fraction after 5-10 min of ischemia, immunoblotting with monoclonal antibodies to the α and β subunits of CaM kinase II indicated that the subunits decreased in the cytosol and increased in the particulate fraction due to ischemia. The decrease in CaM kinase II induced by ischemia correlates with loss of protein in the cytosol. However, in the particulate fraction, loss of activity is probably due to a reversible conformational change. The role of phosphorylation in inactivation and changing the subcellular distribution of the enzyme is being investigated.
ENOLINEDINE AFFORDS NEUROPROTECTION IN A RAT MODEL OF FOCAL ISCHEMIA.

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Various kappa opioid agonists have been shown to be neuroprotective in both global and focal models of cerebral ischemia. The present study evaluated the efficacy of the potent and selective kappa opioid agonist enalaprilat (CI-977) in a rat model of focal cerebral ischemia following continuous subcutaneous administration.

Under general anesthesia the left middle cerebral arterial of male Sprague-Dawley rats was occluded proximal to its lenticulostriate branches by microbipolar coagulation. Enalapriline or vehicle (saline) was administered subcutaneously 30 min before occlusion and by continuous administration starting 5 min post occlusion via an osmotic minipump implanted subcutaneously in the back of the animal. Animals were sacrificed 24 h post occlusion and the brains removed and rapidly stained with haematoxylin-eosin. Areas of infarction were assessed using computer-assisted image analysis and the volume of infarction derived by integration of the known stereotaxic co-ordinates of the 9 planes.

Enalaprilat at 0.1, 0.3, 1.0 mg/kg plus 0.4, 1.2, 4.0 mg/kg/day respectively, dose-dependently decreased the volume of infarction in the cerebral cortex compared to saline control animals. The greatest reduction in infarction (52%) was observed at 1 mg/kg plus 4 mg/kg/day. The study emphasized the potent neuroprotective effect of enalapriline in animal models of cerebral ischemia.


It has been proposed that nitric oxide (NO) synthesized in situ by the constitutive (Ca²⁺-dependent) form of nitric oxide synthase (NOS) may contribute to neurotoxicity associated with cerebral ischemia. We investigated whether inhibition of NO production by N-nitro-L-arginine (NNA) would modify the volume or distribution of infarctions produced by occlusion of the middle cerebral artery (MCA) in spontaneous hypertensive rats. Comparison was made with neuroprotection afforded by electrical stimulation of the cerebral basal ganglia nucleus (FN) (Reis et al. JCBFM 12:53, 1992).

Rats were anesthetized with halothane (2%) and the MCA occluded while either the FN was stimulated or RIL (0.75 mg/kg) or NNA (0.04 µM/kg/min, i.v.) administered for 1 h. Rats were killed 24 h later and infract volumes determined. FN stimulation and RIL reduced infarct volume by 32% (p<0.001; n=6) and hypertensive rats by less than 20%. However, NNA increased volume by 32% (p<0.001; n=6) unrelated to associated hypertension. Repeated administration of NNA (2.4 µM/kg, i.p.) every 3 h increased volume by 32% (p<0.001; n=4) unrelated to associated hypertension.

We conclude that NO generated from constitutive NOS of neurons, glia, endothelium and/or macrophages nor the inducible form of NOS in glia (Galea et al., Soc. Neurosci. Abstr. 1992) or macrophages does not contribute to cell death in focal ischemic infarction.


Antioxidant compounds can reduce CNS damage in both in vitro and in vivo models of hypoxia-ischemia. We have found that 21-aminosteroid antioxidants can reduce neuronal death induced by exposure to glutamate agonists or combined oxygen-glucose deprivation in murine cortical cell cultures.

The water soluble analog of alpha-tocopherol, trolox, produced a 50% reduction in neuronal death induced by exposure to 100 µM glutamate for 30 min. This neuroprotection was increased to 90% by coadministration of 100 µM trolox and 100 µM NMDA.

Intraperitoneal administration of 100 mg/kg of the water soluble analog of alpha-tocopherol, trolox, produced a 50% reduction in mortality in a rat model of acute cerebral ischemia. These findings emphasize the potent neuroprotective effect of enalapriline in animal models of cerebral ischemia.


This study tested the hypothesis that post-ischemic IV administration of acetyl-l-carnitine (ALCAR) can improve cerebral energy metabolism, inhibit free radical-mediated molecular alterations and improve neurological outcome in a clinically relevant model of cardiac arrest (CA) and restoration of spontaneous circulation (ROSC). Seventy adult female beagles were anesthetized with chloralose and divided among 11 experimental groups including those whose dogs were subjected to 10 min of CA followed by 2 min and 2 h of ROSC in the absence and presence of post-ischemic administration of either ALCAR or acetate + carnitine at a concentration of 100 mg/kg immediately following defibrillation and 50 mg/kg every 0.5 h. Although the level of energy deficit as assessed by the acetyl-l-carnitine plus esterified carnitine in the frontal cortex increased by greater than 100% within 30 min following the initiation of either drug treatment relative to vehicle-treated controls only ALCAR-treated animals exhibited a significant amelioration of the abnormally elevated cortical lactate levels and lactate/pyruvate ratios present following 2 h of ROSC.

Free radical-dependent protein oxidation decreased by the presence of dithiobis(hydrazine-reactive) carbonyl groups, is significantly elevated following 2 and 24 h of ROSC in the presence or absence of carnotine + acetate but is not enhanced by treatment with either carnotine + acetate or acetate + carnitine at a concentration of 100 mg/kg immediately following defibrillation and 50 mg/kg every 0.5 h. Although the level of energy deficit as assessed by the acetyl-l-carnitine plus esterified carnitine in the frontal cortex increased by greater than 100% within 30 min following the initiation of either drug treatment relative to vehicle-treated controls only ALCAR-treated animals exhibited a significant amelioration of the abnormally elevated cortical lactate levels and lactate/pyruvate ratios present following 2 h of ROSC.
610.11
Felmate, a novel anti-inflammatory and putative NMDA receptor antagonists, has been shown to reduce infarct injury to the in vivo hippocampal slice and neural necrosis and infarction after hypoxia/ischaemia in the neonatal rat. We tested its neuroprotective properties in an adult rat model of transient complete global cerebral ischemia induced by cardiac arrest. This model gives rise to severe neurological sequelae including neurological extensor hypertonia and severe necrosis to the hippocampus, cortex and Purkinje cell layer of the cerebellum. Felmate was administered to reversible cardiac arrest. Felmate (1000 mg/kg, p.o.) or vehicle (10 ml/kg, H2O) was administered one hour prior to cardiac arrest.
Six days later the animals were given a qualitative neurological examination and were perfused fixed for quantitative histological analysis. Felmate reduced neurological deficits in every neurological category examined. Statistical significance was reached in the total neurological deficit score from 46.7 ± 7.4 to 67.3 ± 7.1 (p<0.05) (the lower the score, the more severe the deficit); facial myoclonus from 1.4 ± 0.2 to 4.1 ± 0.55 (p<0.05) and acoustic startle from 2.50 ± 0.00 to 4.1 ± 0.55 (p<0.05). In addition, Felmate reduced the damage score in the Cortex from 2.0 ± 0.2 to 1.57 ± 0.43 (p<0.05) increased the number of live neurons in the cortex, from 514 ± 44 to 613 ± 127 (p<0.05). No protection was observed in the cerebellum. In conclusion Felmate appears promising as a neuroprotective agent in global forebrain ischemia.

610.12
DELAYED INCREASES IN QUINOLINIC ACID AND SELECTIVE INDUCTION OF KYNURENE PATHWAY ENZYMES AFTER TRANSIENT CEREBRAL ISCHEMIA. K. Saito,1 T. S. Nowak Jr,2 S. P. Markley, and M. P. Hedges1
1NIMH, and Stroke Branch,2 NINDS, Bethesda, MD 20892.
Ac-Quin and the excitotoxin quinoline pathway metabolites, quinolinic acid (QUIN), have been demonstrated in several brain regions following transient cerebral ischemia in the gerbil (J. Cerebral Blood Flow Metabol., 10: 1600, 1990). In the present study, increases in brain indoleamine-2,3-dioxygenase (IDO) activity and QUIN levels occurred 4 days after 10 min ischemia, particularly in hippocampus and, to a lesser extent in striatum, cerebral cortex and thalamus, but not in cerebellum. Notably, these metabolic changes paralleled the degree of neuronal injury, local inflammation and macrophage infiltrates. In addition, the activities of kynurenase, kynurenine 3-hydroxylase and 3-hydroxyanthranilate-3,4-dioxygenase were also increased in hippocampus but not cerebellum. No changes were observed in brain kynurenine aminotransferase activity. Induction of [14C]-QUIN was demonstrated in hippocampus but not cerebellum 1 h after intracranial administration of [14C]L-tryptophan. Increases activities of kynurenine pathway enzymes provide a mechanism to accelerate formation of QUIN from L-tryptophan within the brain. We hypothesize that there are two mechanisms of injury. Studies of the neuroprotective consequences of this delayed QUIN accumulation are warranted.

611.1
INCREASED NGF SYNTHESIS IN RAT VISUAL CORTEX IN RESPONSE TO LIGHT-DEPRIVATION. A.A. Schoups*, R.C. Elliott, W.J. Friedmann and L.B. Black.
Beside their well-described role in the peripheral nervous system, neurotrophins have been shown to be crucial for the development and maintenance of central neurons. To begin investigating their role in activity-dependent plasticity, we measured mRNA's for nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the rat visual system during normal postnatal development and after dark-rearing. Northern blots as well as a fast and highly sensitive solution hybridization technique were used to determine mRNA levels.
In occipital cortex, both NGF mRNA and BDNF mRNA increased during normal development, but were low at 1 week in postnatally, and increased exponentially during the second and third week after birth. Dark-rearing for three weeks following birth resulted in a marked increase in NGF mRNA, confirmed with recent data on a possible role of NGF in experience-dependent plasticity in the visual system. Current experiments involve: 1. Combining immunocytochemistry and in situ hybridization studies, to localize synthesis of neurotrophins and their receptors, and 2. Probing a potential role of glialtames receptors in this experience-dependent modulation of NGF synthesis.
We conclude that occipital cortex responds to light-deprivation by increased synthesis of NGF. Supported by NIH grant # HD 23153 and NS 10259.

611.2
Neurons in the adult cerebral cortex exhibit a high degree of neurochemical diversity. At birth, however, most cortical neurons are immature and they begin to express their specific transmitter only postnatally. Several factors are involved in the determination of neurochemical phenotypes in the cortex? We studied transmission of cortically generated neurons in slice cultures, and in dissociated cell cultures kept in medium with or without serum, from rat cortices at different developmental stages. Cells were stained after different in vitro (DIV) with antibodies against glutamate, GABA, or the neuropeptide VIP. Double-immunofluorescence with the neuronal marker MAP-2 was used to evaluate the percentage of neurons expressing one transmitter. When slice cultures were prepared from postnatal cortices, neurons continued their neurochemical differentiation similar to the development in vivo. In contrast, in slice cultures prepared from E19 cortices, cells that did not complete their migration in vivo did not express their proper transmitter phenotypes, whereas cells that had already reached their final position differentiated properly, indicating that influences during migration of cortical neurons are crucial for neurochemical differentiation. To elucidate the time period during which these influences are required, cortical cells were dissociated either 3 days, 1 day or 1 hour after their birthdate in vivo. Neurons labelled with BrdU 1 day before dissociation were found to contain glutamate or GABA after 7-10 DIV; no neurons labelled with BrdU 1 hour prior to dissociation could be double-labelled with the neurotransmitter antisera even after 14 DIV. Cells labelled with BrdU 1 day before dissociation in an acute slice preparation were also able to express their transmitters properly. These results indicate that factors in the local environment are important for the specification of neurochemical properties of cortical neurons, and that the expression of their transmitter is determined within 24 hours after their birth.

611.3
DEVELOPMENT OF GABA-ERIC SUBPOPULATIONS MARKED BY NEUROPEPTIDES AND CALCIUM-BINDING PROTEINS IN KITTEN VISUAL CORTEX IN VITRO. G. P. Hendrickson and K. H. Tauc.
Cryostat sections of unfixed visual cortex from kittens and cats aged from E16-E18 rat or E6 chick cortex. After a culturing period of 1 to 3 days to one year were used as substrate for cultured embryonic neurons including the elimination of ineffective thalamo-cortical synapses whereas effective projections can form additional contacts. We investigated whether the elimination of the critical period for cortical plasticity is paralleled by changes of the growth permissiveness of the cortical tissue.
Cytosat sections of unfixed visual cortex from kittens and cats aged from 2 weeks to one year were used as substrate for cultured embryonic neurons from E16-E18 rat or E6 chick cortex. After a culturing period of 1 to 3 days viable neurons were labeled with the carboxylfluorescein-ester CFDA-AM and immediately observed with epifluorescence. Neurons readily adhere and grow on white and gray matter of visual cortex from kittens younger than 6 weeks. Thereafter neurons gradually fail to adhere on white matter and growth of neurites is significantly reduced on gray matter. This change in growth behaviour occurs between the sixth and tenth postnatal week, i.e. coincident with the termination of the critical period for cortical malleability.
It is concluded that changes in the growth permissiveness of cortical tissue contribute significantly in the termination of the critical period for cortical plasticity. As the end of the critical period coincides with the time of cortical myelination it is suggested that myelin-associated growth inhibitors (Carrel & Schwab, J.Cell Biol. 106:1281, 1988) may underlie the change in growth permissiveness.

611.4
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During a restricted period of postnatal development the cat visual cortex undergoes an experience-dependent modification of its circuits including the elimination of ineffective thalamo-cortical synapses whereas effective projections can form additional contacts. We investigated whether the termination of the critical period for cortical plasticity is paralleled by changes of the growth permissiveness of the cortical tissue.
Cytosat sections of unfixed visual cortex from kittens and cats aged from 2 weeks to one year were used as substrate for cultured embryonic neurons from E16-E18 rat or E6 chick cortex. After a culturing period of 1 to 3 days viable neurons were labeled with the carboxylfluorescein-ester CFDA-AM and immediately observed with epifluorescence. Neurons readily adhere and grow on white and gray matter of visual cortex from kittens younger than 6 weeks. Thereafter neurons gradually fail to adhere on white matter and growth of neurites is significantly reduced on gray matter. This change in growth behaviour occurs between the sixth and tenth postnatal week, i.e. coincident with the termination of the critical period for cortical malleability.
It is concluded that changes in the growth permissiveness of cortical tissue contribute significantly in the termination of the critical period for cortical plasticity. As the end of the critical period coincides with the time of cortical myelination it is suggested that myelin-associated growth inhibitors (Carrel & Schwab, J.Cell Biol. 106:1281, 1988) may underlie the change in growth permissiveness.

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611.5 THE EMERGENCE OF PINWHEEL-LIKE ORIENTATION DOMAINS IN THE VISUAL CORTEX OF KITTENS DURING THE CRITICAL PERIOD. Tobias Bonhoeffer*, Dae-Shik Kim and Wolf Singer. Max-Planck Institute for Hirnforschung, Deutscherstrasse 46, 6900 Frankfurt 71, FRG.

It has been shown previously that iso-orientation domains in cat visual cortex develop in pinwheel like patterns (Bonhoeffer and Grinvald, Nature 353, 429-431). In the present study we attempted to investigate how these structures develop in the cortex of young kittens. We used optical imaging of intrinsic signals to explore the structure of iso-orientation domains in kitten visual cortex. We developed a technique which allowed us to chronically record intrinsic signals from one animal over a period of 4-8 weeks. Since the intrinsic signals recorded from kitten visual cortex proved to be much stronger than in the technique which allowed us to record activity maps from the intact dura of the animal, we were able to minimize the risk of infection. Using this technique, we found that in kittens of 45 weeks of age pinwheels are already clearly present. Moreover, we also observed strong ocular-dominance maps, allowing us to assess the relationship between ocular-dominance and orientation maps in kittens. The orientation maps changed remarkably little between weeks four and eight postnatally. In the structures from which we were able to acquire optical data (i.e., the parts of area 17/18 on the lateral gyri), we could not observe any new iso-orientation domains or pinwheels being formed during this period of time. This suggests that in normal kittens the basic structure of the orientation maps is formed before the age of 4-5 weeks. We are currently trying to image from the cortex of younger cats, hopefully back to the age of two weeks postnatally.


The clustered horizontal connections of layer 2/3 pyramidal neurons in cat striate cortex specifically link iso-orientation columns. The highly specific pattern of clusters emerges during the first 6 weeks of postnatal development by the selective elimination of unbranched axon collaterals to inappropriate regions and the differential growth of appropriately situated collaterals. Axon rearrangements depend on visual activity: incorrect projections are maintained in animals binocularly deprived for 6 weeks (Callaway & Katz, PNAS 88:745, 1991), and strabismic rearing alters the pattern of clusters (Lowel & Singer, Science 255:209, 1992). Thus, the activity dependence of these intrinsic connections is sensitive to activity cues, animals were deprived of patterned visual experience by binocular lid suture prior to natural eye opening. Eyes were reopened at 6-14 weeks of deprivation to determine whether normal visual experience could restore the normal pattern of clustered connections. Small injections of red fluorescent latex microspheres were made to define patterns of horizontal connections in the deprived eye which should still be refined to the normal pattern by visual experience. Preliminary results indicate that this ability to recover has disappeared by 14 weeks of deprivation. Thus, intrinsic cortical connections, like LGN afferents related to ocular dominance, are sensitive to visual activity during a restricted period of early postnatal life.

611.7 POSTNATAL DEVELOPMENT OF IPSILATERAL CORTICOCORTICAL CONNECTIONS IN THE CAT'S VISUAL CORTEX. D.J. Price, Dept. of Physiology, Univ. Med. Sch., Edinburgh, U.K.

During the postnatal development of connections from area 17 to 18 of the cat's visual cortex, an initially highly exuberant pathway is refined by axonal retraction. Previous studies suggested that the topographic organization of the early immature projection is very crude, but there is no evidence of true axon retraction. In newborn kittens, fluorescent tracer injected into area 18 was not retracted. In young kittens, axonal bifurcation in the area 17 to 18 projection has reached layer 4 and there is no topographically related point in another extrastriate cortical area. Labelling of area 17 was studied. The results reveal a finer organization of the immature area 17 to 18 pathway than was previously suspected. Even at the earliest postnatal ages, while axons from area 17 are just penetrating area 18, there is evidence of rudimentary clusters of association neurones in area 17. Computer-modelling of the distribution of these cells suggests that the majority do not extend axons to topographically related points in other extrastriate cortical areas.

611.8 DEVELOPMENT OF CONNECTIONS IN VISUAL CORTICAL AREAS OF MACAQUE MONKEYS USING CYTOCHROME OXIDASE HISTOCHEMISTRY. M.A. Balsey* & L.C. Hogen. Departments of Physiology & Ophthalmology, UCSC, Santa Cruz, CA 95064.

The development of connections within and between areas V1 and V2 was studied by placing crystals of Di-I in fixed tissue taken from animals at two stages of development: the day of birth and embryonic day 133 (E133). Thirty days after the latest generated neurons in V1 are born (Rakic, '74). At E133 labeled axons from V1 were confined to white matter below V2, where they ramify to some degree, but by birth the V1-V2 projection has reached layer 4. No labeled subplate neurons were seen after dye injections at either age. Intrinsic V1 connections are quite extensive at E133; the same layers are involved at this stage as at maturity, and extend approximately the same distances, with the exception of layer 1, where fibers extend over a larger homotopic region. In the feedback direction, the V2 to V1 projection has entered the gray matter by E133. At this stage the projection arises principally from deep layer cells, each of which has a dendrite which extends into layer 1. At birth the V2 to V1 projection is already predominantly from superficial layer cells. These observations suggest that the modular arrangement of connections in superficial layers of V1 is sculpted from a more uniform state; and that the feedback projection begins to inervate its target before the forward projection does so.

611.9 NATURALLY-STRABISMIC PRIMATE LACKS INTRINSIC HORIZONTAL CONNECTIONS FOR BINOUCULAR VISION IN STRIATE CORTEX. L. Teschan, R. F. Hardin, L. Oral, and J. B. Shepherd. The Hospital, Neurosurgery, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Although it has been postulated that strabismus in human infants is associated with abnormalities in connections in the visual cortex, no anatomic data from naturally-strabismic primates has been reported. We have now shown abnormalities of horizontal connections in visual area V1 in a macaque with infantile-onset strabismus. The monkey developed esotropia 6 weeks after birth and showed ocular motor behaviors that characterize early-onset esotropia in human infants. In V1 of a normal primate, injections of horseradish peroxidase into the left occipital lobe were processed for either cytochrome oxidase or autoradiography. In the amblyopic eye, but remarkably, cytochrome oxidase (CO) activity in layer IV of normal primates was quite extensive at E133; the same layers are correlated input from the 2 eyes is wide, pale columns. Comparison with adjacent autoradiographs established that the pattern of columns was quite homogeneous. In layers II,III alternating light and dark rows of blobs were visible. The light rows fit in register with the V1/V2 border. However, at E133 there is no evidence of clusters of connections in superficial layers of V1, whereas these columns are quite extensive at birth. These findings indicate that early monocular suture in macaques produces uniform distribution of cytochrome oxidase activity in layer IV of striate cortex, but visual deprivation combined with subsequent enucleation results in an abnormal pattern of Cytochrome oxidase activity in layer IV of amblyopic primate cortex.

611.10 LABELING OF OCULAR DOMINANCE COLUMNS IN AMBLIOPIQUE MACAQUE MONKEYS USING CYTOCHROME OXIDASE HISTOCHEMISTRY. M.A. Balsey*, L.C. Hogue. Departments of Physiology & Ophthalmology, UCSC, Santa Cruz, CA 94143-0444.

In two monkeys undergoing suture of the right eyelids at 1 week of age, [3H]-proline was injected into single geniculate lamina. Autoradiographs revealed shrunken ocular dominance columns belonging to the amblioptic eye, but remarkably, cytochrome oxidase (CO) activity in layer IV appeared completely homogenous. In layers II,III alternating dark and light rows of columns were visible. The light rows fit in register with deprived eye dominance columns in V1. To label the eye dominance columns in V1 using CO, a third monkey was raised with the right eyelids sutured open. Eyes were reopened at after 6-14 weeks of deprivation. Thus, intrinsic cortical connections, like LGN afferents related to ocular dominance, are sensitive to visual activity during a restricted period of early postnatal life. Supported by NIH grant EY07960 and the L.P. Markey Charitable Trust.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992

In macaques raised with unilateral eyelid suture, cytochrome oxidase activity is homogenous in layer IVC of striate cortex (Stryker & Horton, 1992). Subsequent enucleation of the normal eye causes thin, dark columns alternating with white columns to appear in layer IVC. The thin, dark columns are the ocular dominance columns of the amblyopic eye. In an analogous clinical case, we examined the pattern of cytochrome oxidase activity in a 53-year-old man with amblyopia in the left eye (20/400 acuity), who became blind in his normal right eye 3 months before death. The amblyopia was due to a difference of 6 diopters in the refractive power of the two eyes (anisometropia), first detected at age 5.

The mosaic of ocular dominance columns in each striate cortex was reconstructed from serial flattened sections reacted for cytochrome oxidase activity. Surprisingly, no shrinkage of the ocular dominance columns serving the amblyopic eye was observed. Dark stripes occupied 48% of the column area in the left cortex and 54% of the column area in the right cortex. Slightly more cortex was filled in each side by the contralateral eye, reflecting the normal attenuation of ipsilateral eye columns in peripheral binocular cortex. We conclude that ocular dominance columns in layer IVC of human striate cortex do not shrink in anisometropia, implying a different cortical basis for this form of amblyopia.

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612.1 PRENATAL COCAINE EXPOSURE ALTERS DOPAMINE TRANSPORTER BINDING IN ADULT MICE. Q.A. Pritchard*, J.J. Byrnes, L.G. Miller, Dept. of Pharmacology and Experimental Therapeutics and Neurosciences Program, Tufts Univ. School of Medicine, Boston, MA 02111.

Prenatal cocaine administration is associated with persistent behavioral effects, but the neurochemical basis for these effects is uncertain. We exposed pregnant mice to cocaine, 10 mg/kg, during days 14-21 of gestation. At 6 weeks of age, offspring were evaluated for motor activity and dopamine transporter binding in striatum in vivo using WIN 35,428. No differences in motor activity were observed among untreated, vehicle-exposed, or cocaine-exposed mice. Similarly, no change in activity was noted in response to chronic cocaine among the exposure groups. However, total dopamine transporter binding in striatum was significantly reduced by approximately 20% in cocaine-exposed mice compared to the other groups. No change was observed in nonspecific binding evaluated in cerebellum. Specific binding in striatum was thus reduced significantly by cocaine. These data indicate that prenatal cocaine exposure is not associated with changes in motor activity untreated or in response to chronic cocaine, but is associated with alterations in dopamine transporter binding in mature offspring.

612.2 REGULATION OF DOPAMINE RECEPTORS mRNA EXPRESSION IN THE RAT BRAIN BY COCAINE. C. Spyra(1), A. Fritzhauer(1), G.W. Humble(1) and S.C. Sallow(1,2). 1. Fisberg Center for Research for Neurobiology and 2. Department of Neurology, The Mount Sinai Medical Center, New York, NY, 10029, 3. Lab. of Pharmacology, Medical School, University of Crete, Greece.

The mesolimbic/mesocortical D_Aergic pathways and the DA receptor subtypes, pharmacologically defined as D1/D2, appear to mediate the reinforcing properties of cocaine, on both behavioural and biochemical grounds. The present study focuses on possible regulation of D1 and D2 receptor gene expression in rat brain by cocaine treatment, using in situ hybridization (ISH). Adult male rats were treated with intraperitoneal cocaine administration (0; 5.0; 10.0; 20.0; 40.0 mg/kg) or for 15 days (0; 10.0; 20 mg/kg). Twenty four hrs after the last injection, the animals were deeply anaesthetized and perfused. Their brains were frozen and 25mum thick sections were taken through the accumbens,striatum and midbrain with a sliding microtome. The floating sections were processed for ISH with 35S-radiolabeled rat D1 and D2 mRNA probes. Quantitative ISH, performed on film autoradiograms with a computer assisted densitometer, was used to examine changes in the levels of D1 and D2 mRNA in nucleus accumbens (ACC), olfactory tubercle (TO), caudate nucleus (CP), ventral tegmental area (VTA) and substantia nigra(SN). The results show that D1 mRNA expression was affected by neither acute nor chronic cocaine treatment at the ACC, TO and CP. Similarly no changes were revealed in the D2 mRNA levels at the ACC, CP, and SN. An average of 25% decrease (statistical significance 95%) in D2 mRNA density was measured at TO in animals treated with single injections of cocaine at the high doses(20 and 40 mg/kg). A tendency for decrease with the same regimen was observed at VTA. Within the limits of the expensive paradigm used in this study, the data suggest that the regulation of DA receptor gene expression by cocaine is not robust and manifests dose, receptor subtype and anatomical specificity. (Supported by the Aaron Diamond Foundation)
DRUGS OF ABUSE: COCAINE AND OTHER STIMULANTS

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Several studies have suggested that certain calcium channel modulators may affect the behavioral effects of cocaine. The present study has examined the effects of various ion channel modulators directly upon the binding of cocaine to the dopamine transporter. The effects of NaCl, KCl, and CaCl2 and their respective calcium channel modulators had upon the striatal cocaine binding site were determined using the cocaine analog [3H]WIN 35,428. The addition of NaCl had no effect upon specific binding however, CaCl2 and to a lesser extent KCI decreased binding. Various Na+ channel blockers were also tested for their ability to inhibit specific [3H]WIN 35,428 binding. Most of the Na+ channel blockers tested were of moderate potency, the exceptions being benzamid and flunarizine which displayed higher potency. Both of these agents are also reported to have activity at the Ca2+ channel. The K+ channel blockers were of low and moderate potency while the CI channel blockers had no effect. Of the Ca2+ channel blockers tested only pirenzepine demonstrated high potency. This was postulated to be due to its ability to act upon both L and T-type channels. These results suggest that the Ca2+ blockers in cocaine depend further study as useful therapeutic potential in the treatment of cocaine addiction.

612.7 NEUROANATOMIC AND MOLECULAR SPECIFICITY OF C-FOS INDUCTION BY COCAINE IN DEVELOPING RAT BRAIN. Barry E. Krasowski* and Steven E. Hyman. Molecular Neurobiology Laboratory, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.

A class of immediate early genes (e.g., the IEG c-fos) that act as transcription factors, may subserve one mechanism by which substances of abuse alter programs of neural gene expression. We have examined injected saline (vehicle control) or cocaine (30 mg/kg, ip) in male rats (P9, P15, P28) and adults and sacrificed animals acutely (45 minutes for mRNA and 24 hours for histone hyperphosphorylation analysis) and 120 minutes for immunohistochemical analysis to determine the ontogeny and spatial distribution of c-Fos expression. In P9 rats, cocaine increased expression of c-Fos in striatal patches, a few scattered globus pallidus neurons, in layer VI neocortical neurons, but not in anterior cingular cortex. In P15, cocaine induced striatal c-Fos is more diffuse, with a lateral predominance. In globus pallidus there is a high density of c-Fos immunoreactive (IR) neurons. In neocortex there is a significance of C-fos expression also observed in the thalamus, particularly evident in infragranular layers. Anterior cingular cortex, which is minimally activated on P8 or P15, has a high density of c-Fos IR neurons in cocaine exposed P28 and adult animals. Changes in either pre- or postsynaptic 5-HT1A receptors. The effects of NaCl, KCl, and CaCl2 and their respective calcium channel modulators had upon the striatal cocaine binding site were determined using the cocaine analog [3H]WIN 35,428. The addition of NaCl had no effect upon specific binding however, CaCl2 and to a lesser extent KCI decreased binding. Various Na+ channel blockers were also tested for their ability to inhibit specific [3H]WIN 35,428 binding. Most of the Na+ channel blockers tested were of moderate potency, the exceptions being benzamid and flunarizine which displayed higher potency. Both of these agents are also reported to have activity at the Ca2+ channel. The K+ channel blockers were of low and moderate potency while the CI channel blockers had no effect. Of the Ca2+ channel blockers tested only pirenzepine demonstrated high potency. This was postulated to be due to its ability to act upon both L and T-type channels. These results suggest that the Ca2+ blockers in cocaine depend further study as useful therapeutic potential in the treatment of cocaine addiction.


Repeated administration of cocaine causes augmentation in behavioral effects (behavioral sensitization) induced by acute administration. However, the specific adaptive neurochemical mechanisms underlying this behavioral sensitization are not well-understood. Electrophysiological studies have suggested that the effects of cocaine on serotonin function may be modulated by 5-HT1A receptor subtype. In the present studies we examined the changes in the binding of [3H]8-hydroxy-2-(diphenylamino)tetralin (8-OH-DPAT, 5-HT1A selective ligand) in rat cortex and hippocampus after repeated cocaine administration. Cocaine (10 mg/kg) was administered once daily by intraperitoneal injection for a total of 9 injections over 11 days (5 injections with 2 days off, followed by 4 injections). Four groups [ saline (S); cocaine (C); saline (S); cocaine (C)] of animals were pretreated with 8 injections of S or C followed by the 9th injection as S or C "challenge". The animals were sacrificed by decapitation 24 hours after the last injection, brains removed and dissected into cortices and hippocampal regions for [3H]-8-OH-DPAT binding. There were no statistically significant differences in either the number of binding sites (Bmax) or the affinity (Kd) of DPAT binding in cortex and hippocampus from various groups. These results suggest that behavioral sensitization with repeated cocaine administration is not associated with changes in either pre- or postsynaptic 5-HT1A receptors.

Amphetamine derivatives and other substrates of the catecholamine transporter such as tyramine are believed to induce release of dopamine via a "monomodal exchange diffusion". Uptake of amphetamine derivatives by the catecholamine transporter is thought to be coupled with a non-exocytic release of dopamine. An important assumption in this study is that specificity of these agents is derived from their ability to act as substrates of the dopamine transporter. Therefore, a good correlation is expected between the competitive inhibition of dopamine uptake and dopamine release. Phentolamine (PHE) (PC12) cells have been used extensively to characterize the exocytic release of dopamine and we have begun to study PC12 cells as a model of non-exocytic dopamine release.

Our findings on the stereospecific release of dopamine from PC12 cells by amphetamine derivatives is supported by earlier reports in the literature. It is believed to release dopamine via a carboxylated mechanism. Unlike, consistence with the non-exocytic exchange diffusion model, the ability of amphetamine derivatives to release dopamine in calcium-independent is shown. However, there appears to be a poor correlation between the inhibition of dopamine reuptake and the ability to induce release. We have observed a rank order for fenfluramine > d-amphetamine > l-amphetamine > tyramine in the ability to release dopamine and d-amphetamine = l-amphetamine > tyramine = fenfluramine for inhibition of uptake. Inasmuch as tyramine has also been reported to bind to and be a substrate of the vesicular dopamine transporter, we examined whether the transport of catecholamines into storage vesicles is a potential source of specificity for dopamine release. An identical rank order was observed in the ability of amphetamine derivatives to inhibit [(3H)DA] neurotransmitter release to vesicles as was earlier found to release dopamine. These results suggest that the vesicular transport may play a critical role in the mechanism of amphetamine-induced dopamine release from PC12 cells.

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612.12 DAILY POST-SESSION COCAINE ADMINISTRATION IMPAIRS ACQUISITION OF A POSITIVELY-MOTIVATED AUTOSHAPED LEVER-TOUCH RESPONSE IN RATS. P.H. Janak*, W.A. Rodriguez and J.L. Martinez. Dept. of Psychology, Univ. of California, Berkeley, CA 94720.

The effects of daily peripheral (IP) post-session injection of cocaine (COC) on the development of an autoshaped lever-touch response were investigated. Male Sprague-Dawley rats received 10 daily pairings of a retractable lever (CS) and food delivery (US). Reinforcement delivery was contingent on lever being touched. If the subjects contacted the lever during CS presentation, then reinforcement was delivered immediately. COC, at a dose of 5.5 mg/kg [F(1,30) = 5.21, p < 0.05], but not 2.5 mg/kg [F(1,30) = 0.18, p > 0.5], impaired acquisition of the lever-touch response. COC's effect on lever-touch acquisition depended upon the time of drug administration relative to the conditioning session, as injection of COC 3 hrs after each session did not affect response acquisition. In addition, COC's effect depended on explicit CS-US pairing as post-session COC administration did not alter responding when the presentation of both the CS and the US were uncorrelated. These results indicate that the post-session administration of COC can alter the retention of a positively-motivated conditioned response, as seen by impaired acquisition of the lever-touch response. (Supported by DA06192 and DA05757.)
613.5 THE 3-BENZODIAZEPINE FYK52466 SELECTIVELY BLOCKS AMPA/KAINATE RECEPTORS BY A NOVEL, NON-COMPETITIVE MECHANISM. S.D. Donevan* and M.A. Rogawski. Erythropoiesis Research Branch, NNDS, NIH, Bethesda, MD 20892.

Excessive activation of non-NMDA (AMPA/kainate) excitatory amino acid receptors may play a role in the pathogenesis of epilepsy and various neurodegenerative disorders. Recent studies have demonstrated that the 3-benzodiazepine FYK52466 antagonizes non-NMDA excitatory amino acid receptor responses (Tarrawa et al., 1990; Quartiai and Durand, 1991). In the present study we compared the effects of FYK52466 with that of the quinoxaline NBQX on currents evoked by AMPA and kainate in whole cell recordings from cultured rat hippocampal neurons. FYK52466 caused a concentration-dependent block of inward currents evoked by AMPA (EC50=7.9+0.4 µM) and kainate (11±1 µM), but was inactive against currents evoked by NMDA or GABA. The EC50 values for kainate in the presence of 10 and 30 µM FYK52466 were 69±2 µM and 202±18 µM, respectively. Similarly were similar to control (173±16 µM), whereas KYK 52466 produced a concentration-dependent reduction in the maximal current evoked by kainate. In contrast, NBQX caused a concentration-dependent rightward shift in the kainate concentration-response curve with no change in the maximal response to kainate. Thus FYK52466 is a non-selective antagonist, whereas NBQX acts in a competitive fashion at the AMPA/kainate site. FYK52466 did not appear to block via an open channel mechanism as there was no voltage-or use-dependence to its actions. These results demonstrate that FYK52466 selectively inhibits AMPA/kainate responses by a novel non-competitive mechanism. Non-selective non-NMDA antagonists such as KYK52466 could offer advantages in the treatment of neurological disorders, particularly in situations where high levels of glutamate would render the competitive antagonists relatively ineffective.

613.7 NMDA RECEPTOR SPlice VARIANTS DIFFER IN THEIR RESPONSE TO ETHANOL. S.B. Treistman, B. Bayley, V.V. Koltchik, and V. Anantharam. Dept. of Pharmacology, Univ. of Mass. Medical School, Worcester, MA 01655 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

A number of laboratories have reported the NMDA receptor to be very sensitive to ethanol, and the reduction of current through this receptor/channel may underlie some of the behavioral effects of ethanol. Using RT-PCR, we have isolated four splice variants of the NMDA receptor from rat brain (see V. Anantharam abstract, this meeting). The variants, in each of which two cassette (21 and 37 amino acids) are present or absent, have been expressed in Xenopus oocytes, and their response to ethanol has been examined. In all of the splice variants 25-100 µM ethanol reduces the inward current evoked by 100 µM kainate. However, the magnitude of this reduction differs among the different variants. The rank-ordering of the variants according to the reduction of the inward current is kainate, with the most sensitive first, is: NMDAR1-LL > NMDAR1-LS > NMDAR1-LSL > NMDAR1-SS. In addition to differences in their ethanol sensitivity, we find that the apparent desensitization of the variants differs, with NMDAR1-LL showing significantly greater desensitization than NMDAR1-SS. We are currently determining the relationship between the apparent desensitization of the receptor and its response to ethanol. Work supported by AA120 grant AA05542.

613.8 NON-SELECTIVE ACTIONS OF ETHANOL AS AN EXCITATORY AMINO ACID (EAA) ANTAGONIST ON RAT SPINAL NEURONES IN VIVO. D. Lodge*, S.N. Davies and M.G. Jonas, Royal Veterinary College, London NW1, UK.

Recent studies have focussed the on the non-selective, ethanol-induced reduction of both the potentiating and blocking subtypes of central glutamate receptors. Pharmacological characterization of the action of ethanol on the non-NMDA antagonist AMPA (2-amino-5-phosphonovalerate) on glutamate receptors was investigated in Langendorff perfused brain slices. In situ hybridization was performed on coronal sections from control and ethanol injected rats. The IC50 values for AMPA (10 µM) in the presence of 100 µM ethanol were determined using a computer-assisted digital analysis of silver grain distribution over the glycine and kainate receptors. The presence of AMPA receptors were found to have different effects on AMPA receptors: (1) currents elicited by low concentrations of AMPA (10 µM) were inhibited by ethanol with an IC50 value of 180 ±19 µM and (2) currents elicited by high concentrations of AMPA (100 µM) were potentiated with an EC50 value of 88 ±22 µM. The maximal potency effect of ethanol on AMPA currents was around 170%. Quantitative analysis showed that the apparent Kd value for AMPA with respect to its potentiation of AMPA responses was about 15-fold lower that its Kd value for inhibition of AMPA responses. This suggests that the ability of ethanol to potentiate or inhibit AMPA responses may be mediated by different sites. Nonetheless, the two opposing effects of AMPA on AMPA responses are specific for the L-configuration of AMPA. This unusual agonistic/antagonistic property of AMPA may explain its unusual properties with regard to antagonism of non-NMDA receptor mediated events previously described.

613.9 AGENTS WHICH ANTAGONIZE THE NMDA RECEPTOR-CHANNEL COMPLEX IN VIVO ALSO CAUSE DISTURBANCES OF MOTOR COORDINATION. R.M. Muller, M.D. Pardridge, and R. Muller. Dept. of Pharmacology, Boehringer Ingelheim R, W-6507 Ingelheim, Germany.

We have investigated the relationship between functional antagonism of the NMDA receptor-channel complex in vivo and disturbances of motor coordination. Antagonism of the NMDA receptor-channel complex was assessed by measuring the ability of various compounds to inhibit NMDA-induced lethality in mice. Disturbances of motor coordination were measured by the rotated technique. Noncompetitive NMDA antagonists of both arylcyclohexamine type ((+)-MK-801, (-)-MK-801, ketamine) and benzodiazepine type (diazepam) blocked lethality after systemic administration of doses up to 100 mg/kg and did not interfere with motor coordination. We conclude that any compound which antagonizes the NMDA receptor in vivo irrespective of whether it is a noncompetitive, competitive or glycine site antagonist, will disturb motor coordination.


Non-competitive NMDA antagonists, such as PCP, are thought to produce a schizophrenia-like syndrome in humans. This hypothesis was formally evaluated in humans using ketamine. METHODS: Healthy subjects (n=18) completed 3 test days in a randomized order, under double-blind conditions: placebo, ketamine 0.1 mg/kg, ketamine 0.5 mg/kg. All drugs were infused I.V. over 2 minutes.

RESULTS: The Mini-Mental Status Examination was not affected by either ketamine dose. Positive and negative symptoms of schizophrenia assessed by the Brief Psychiatric Rating Scale, the Yale-Brown-Cornellman Scale and the Perceptual Aberration subscale of the Psychosis Proneness scale, and dissociative symptoms assessed by the Clinician Administered Dissociation Scale, all showed dose-related increases. Frontal lobe function assessed by Wisconsin Card Sort perseverative errors and by assessment of verbal fluency showed dose-related impairment. Ketamine also produced dose-dependent impairment in delayed but not immediate recall of object names. Ketamine produced dose-dependent increased plasma cortisol and prolactin and suppressed plasma HVA. IMPLICATIONS: Ketamine transiently produced a broad range of the symptoms and neuropsychological deficits characteristic of schizophrenic patients. These data place further emphasis of evaluations of excitatory amino acid function in schizophrenia.
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613.11 SOMATOSTATIN CONTAINING DENTATE HILAR NEURONS IN PRIMARY CULTURE EXPRESS Ca2+-PERMEABLE KAINATE RECEPTORS. Simon J. Gibbons and Richard J. Miller, Dept Pharmacol. and Physiol. Sci., University of Chicago, 942 East 58th St., Chicago, IL 60637, USA.

We have studied the excitatory amino acid receptor pharmacology in a population of hippocampal OABA-containing neurons in primary culture. Neurons were dissociated from the dentate gyrus of 5 day old rats and cultured in a defined serum-free medium over a feeding layer of astrocytes. These cultures are highly enriched (85%) in neurons which express immunoreactivity for somatostatin-14 and OABA but not the Ca2+-binding proteins parvalbumin or calbindin D28K. Together with the morphological appearance of the cells, this information suggests that they represent a population of neurons which are particularly sensitive to inositol 1,4,5-trisphosphate (IP3) and caffeine.

613.12 NMDA DIFFERENTIALLY STIMULATES SOMATOSTATIN (SS) BUT NOT NEUROPEPTIDE Y (NPY) GENE EXPRESSION IN CORTICAL SS/NPY PRODUCING NEURONES. Yu.C. Patel, A. Warzynska, G. Kent, L.-L. Liu, D.N. Papadimitriou, and S.C. Patel, Fraser Labs, McGill University, Montreal, Quebec, and Newington VAMC, CT.

SS and NPY are coproduced in a subpopulation of neurons that are selectively resistant to NMDA neurotoxicity. We have previously reported that quinolinic acid (QA) and NMDA augment SS-mRNA in cultured fetal rat cortical neurons. Here we have examined coregulation of SS and NPY gene expression by this system and compared their effects with those of forskolin (F) and PMA known to activate SS and NPY gene transcription by cAMP or protein kinase-C (PKC)-dependent mechanisms. Cultures were treated with different agents for up to 24 h. SS-mRNA was determined by Northern analysis with cRNA probes.

SS-mRNA (fold increase) NPY mRNA (fold increase)

<table>
<thead>
<tr>
<th>Agent</th>
<th>SS-mRNA</th>
<th>NPY mRNA</th>
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<tbody>
<tr>
<td>QA 5 mM</td>
<td>3.8*</td>
<td></td>
</tr>
<tr>
<td>NMDA 5 mM</td>
<td>5.0*</td>
<td>0.65</td>
</tr>
<tr>
<td>forskolin 10 uM</td>
<td>4.5*</td>
<td>vs control 1.6*</td>
</tr>
<tr>
<td>PMA 0.4 uM</td>
<td>1.5*</td>
<td>2.9*</td>
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| NMDA and stimulated mRNA for SS but not NPY mRNA. In contrast, F and PMA augmented both SS and NPY mRNA.


614.1 MAPPING OF CYTOSOLIC CALCIUM CONCENTRATION IN GROWING AXONS OF RAT SENSORY NEURONES. S.R. Bolsover, M.R. Duchen* and A. Amato. Physiology Department, University College London.

Previous work using the calcium indicator Fura-2 has suggested the presence of steady-state gradients of cytosolic free calcium concentration ([Ca2+]i) in various types of nerve cells that are extending axons or dendrites. These measurements were subject to possible errors caused by uptake of Fura-2 into intracellular organelles. We have therefore used dextran-conjugated Fura-2, which is not taken up into organelles, to measure [Ca2+]i in the axons and growth cones of sensory neurones in culture.

After injection with Fura-2 dextran (MW = 10,000, Molecular Probes) dorsal root ganglion cells from adult rats were incubated in Hams F12 medium supplemented with 4% Ultraser G (Gibco) in an atmosphere of 5% CO2 and 95% air. [Ca2+]i was imaged in 31 growth cones, where it was a steady-state concentration, spontaneous or induced by A23187, caused growth cone retraction. Increases in [Ca2+]i were observed (EC50 values for AMPA = 11.5 ± 3.3 µM, NMDA = 5.6 ± 1.7 µM). A23187 increased [Ca2+]i by an average of 240±30nM, n=10, while AMPA and NMDA increased [Ca2+]i by 304±35nM and 298±30nM, respectively.

614.2 SPATIAL GRADIENTS OF CYTOSOLIC CALCIUM DURING DEPOLARIZATION OF DEVELOPING SENSORY NEURONES. F.A. Al-Mohanna, A. Amato and S.R. Bolsover*, Physiology Department, University College London

Spatial gradients of cytosolic calcium concentration ([Ca2+]i) were observed along the axon (soma = 225±44nM, n=31). No steady-state gradients were observed in the growth cone, and a small number of spatially restricted hotspots were observed. We have now examined whether similar [Ca2+]i gradients are set up by depolarization of primary sensory neurones. As before, we used the failure of Fura-2 fluorescence during steady illumination with 380nm light to measure [Ca2+]i with a time resolution of 60ms.

After the first day after plating on a laminin substrate, dorsal root ganglion cells isolated from adult rats extended large (up to 40µm across) growth cones connected to the cell body by broad, short neurites. When cells at this early stage of axon outgrowth were whole-cell patch clamped and depolarized, [Ca2+]i increased in all regions of the cell, so that no spatial gradients of [Ca2+]i were detectable in the neurite and growth cones.

After two or more days in culture, cells exhibited the more familiar appearance of long narrow axons terminated by small (up to 20µm across) growth cones. Since these cells are not suitable for voltage clamp, we examined [Ca2+]i changes during a train of five action potentials delivered during a 50ms period. Unlike the situation in the early growth cones, depolarization produced a clear [Ca2+]i gradient in the growth cones, with the leading edge of the growth cone showing a greater [Ca2+]i increase than the more proximal regions of the growth cone. These results raise the possibility that electrical activity could selectively activate calcium-dependent processes at the growth cone leading edge.


614.3 GROWTH CONE COLLAPSE, A DETERMINANT OF SYNAPSE SPECIFICITY. N.I. Syed*, K. Laitiawik and A.G.M. Bullough, Departments of Physiology and Anatomy, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

A model for system by examining synaptogenesis is provided by the large, identified dopamine neurone (RPD1) of the adult mollusc Lymnaea stagnalis. (Ca2+ in neurons exhibits robust sprouting and synaptogenesis with appropriate targets in vitro. Behavioral analysis of the growth cone from both RPD1 and the appropriate targets indicates mutual attraction. We present evidence that this attraction is mediated by target RPD1 growth cones prior to synaptogenesis is mediated via transmitter/receptor interaction. We also demonstrate that RPD1 cell growth cones contain dopamine and that the target cell growth cones respond selectively to the exogenous application of dopamine.

However, when cultured with inappropriate target cells RPD1 does not form synapses, rather its growth cone induces the collapse of approaching target growth cones, and its own growth cone is unstable in the presence of extracellular contact. Thus the possibility that electrical activity could selectively activate calcium-dependent processes at the growth cone leading edge.


614.4 DEPOLARIZATION TRIGGERED NEURITE RETRACTION OF LEECH CELLS IN CULTURE IS SUBSTRATE AND CALCIUM DEPENDENT. M.D. Neely* and M. Gerszten, Blooener Univ. Basel, 4056 Basel, Switzerland.

The substrate upon which a leech neurone is placed influences not only its pattern of growth and the distribution of Ca2+ channels on its surface, but also the degree of neurite outgrowth and synaptogenesis is mediated by transmitter/receptor interaction. We also demonstrate that RPD1 cell growth cones contain dopamine and that the target cell growth cones respond selectively to the exogenous application of dopamine.

However, when cultured with inappropriate target cells RPD1 does not form synapses, rather its growth cone induces the collapse of approaching target growth cones, and its own growth cone is unstable in the presence of extracellular contact. Thus the possibility that electrical activity could selectively activate calcium-dependent processes at the growth cone leading edge.


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5.14.5

SERUM LYSOPHOSPHATIDATES CAUSE RECEPTOR MEDIATED NEURITE RETRACTION IN PC12 CELLS. G. Tikvah, D. L. Dyer and R. Mitke*. Lab. of Cellular and Molecular Neurobiology, Dept. of Physiology, University of California, Irvine, CA 92717 Lysophosphatidic acids (LPA's), produced during blood coagulation and bound to serum albumin were found to be the principal growth inhibitory component causing neurite retraction in NFG differentiated PC12 cells. LPA's purified from albumin, or of synthetic origin, elicited neurite retraction with an ED50 in the micromolar range. Application of LPA's caused the intracellular level of IP3 to increase >10 fold within 7.5 min. Monitoring intracellular free [Ca++] levels with Fura2 ratio-imaging revealed a concomitant transient increase from the resting level (<100nM) to 300 nM. Neurite retraction was abolished in the presence of extracellular Ca++. Divalent cation blockers of Ca++ channels had similar effect; however, organic blockers of the different classes of voltage gated Ca++ channels were ineffective. This suggests the involvement of a non-voltage activated Ca++-influx mechanism. Bradykinin and carbachol activated similar second messenger events without causing neurite retraction. Thus, increased P2Z turnover and a transient rise in Ca++ are just sufficient for neurite retraction by LPA.

Neurite retraction was also prevented by pretreatment with chelerythrine, supporting the involvement of a Ca++-mediated, CAM-dependent pathway. It is concluded that LPA-induced neurite retraction is not due to an increase in [Ca++] alone, but depends on interactions of different second messenger pathways.

Supported by grants BNS-9010398 and NS-23284.

5.14.6


Fasciclin II is a neural cell adhesion molecule that is structurally similar to NCAM and is expressed by the T1 neuron precursor axons during axonmatogenesis. Previously, we showed that chromosome assisted laser inactivation (CALI) directed against fasciclin II results in a perturbation of axonomegasy but not axon adhesion of the T1 retinal neurons (Ishihara et al., Nature 350: 441-444, 1991). Recently, single cell CALI has been developed, in which the laser is focused on one cell (Sydor et al., Neuron, 17: 14, 1994). We now show that single cell CALI against fasciclin II on the T1 neurons perturbs axonomegasy with high efficacy and reveals a three-hour time period during which CALI will cause this perturbation.

Laser irradiation of T1 neurons in whole mounted grasshopper embryos at the 30% stage of development incubated with matalich-green labeled anti-fasciclin II blocked axon formation (15/18). No significant disruption in axonomegasy was seen in non-irradiated contralateral limbs (n=7), or in embryos incubated with dye-labeled anti-fasciclin I regardless of laser irradiation (n=15). Single-cell CALI against fasciclin II had no effect on axon adhesion (n=20), in contrast with CALI against fasciclin I. Also, single cell CALI against fasciclin II was only effective between complete T1 cell emergence from the epithelium and these cells becoming tear-shaped. This stage is coincident with the localisation of cytoskeletal elements and organelles to the proximal pole from which the growth cone will emerge (Lefebvre and Bentley, J. Cell Biol. 106: 1737, 1989). The higher efficacy of single cell CALI over large scale CALI in perturbing axonomegasy may be due to greater antibody accessibility, more precise aiming of the laser, and the ability to perform CALI in precise stages based on T1 cell morphology. This last feature also enabled us to pinpoint the narrow time window during which CALI is effective in perturbing axonomegasy.

5.14.7


Glycosaminolylglycans (GAG) are high molecular weight, acidic polysaccharides, which are important structural components of the extracellular matrix (ECM). Galectin-III is a relatively non-selective, homotypic cell-surface recognition receptor for sulfated GAG (Bender et al., J. Cell Biol. 108: 1737, 1989). Galectin-III was found to be expressed by the developing retinal ganglion cell bodies, and it is at the surface that Galectin-III exerts its effects on the localisation of axon growth. Galectin-III has been shown to bind to GAG (Keller et al., J. Cell Biol. 102: 1230, 1986). The pattern of Galectin-III expression was found to correlate with the boundary of the developing retina, which is also a boundary of the expression of GAG (Keller et al., J. Cell Biol. 102: 1230, 1986). The pattern of Galectin-III expression was found to correlate with the boundary of the developing retina, which is also a boundary of the expression of GAG (Keller et al., J. Cell Biol. 102: 1230, 1986).

5.14.8


NCAM, N-cadherin and L1 are cell adhesion molecules now known to be important for promoting axon growth from projection neurons, e.g. retinal ganglion cells. However, the CAMs important for regulating axon outgrowth from non-projection neurons, like amacrine and photoreceptor cells, are not known. Such local circuit neurons extend their neurites on cellular surfaces not normally encountered by the ganglion cell axons, to terminate on neighboring cells. Therefore, we compared neurite outgrowth from rat ganglion cells, amacrine cells and rods in vitro on immunopurified forms of NCAM, L1 and N-cadherin. Single isolated early postnatal ganglion cells grow neurites on all three cell adhesion molecules. NCAM and N-cadherin strongly promote neurite outgrowth from either early (P3) or late (P10) postnatal amacrine cells, while L1 promotes neurite outgrowth from only a small percentage of the amacrine cells. None of these cell adhesion molecules support neurite outgrowth from postnatal rods, but, surprisingly, NCAM stimulated vigorous neurite extension from rods isolated from postnatal day 10. These results show that NCAM, N-cadherin, and L1 can promote neurite outgrowth from local circuit neurons, but the use of any particular CAM is dependent on the cell type and the developmental period. Supported by NIH NS 30305; Sigma Xi.

5.14.9

PATTERNS OF GROWTH CONE CONTACT WITH DIFFERENT ADHESIVE SUBSTANCES AS DETERMINED BY INTERFERENCE REFLECTION MICROSCOPY. J.A. Drazba*, C.L. Smith, and Y. Lemmon. Lab of Neurobiology, Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892 and Dept. of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH, 44106.

We examined dynamic changes in the closeness of growth cone contacts with laminin, L1/D9, or N-cadherin fibroblast substrates by measuring reflectance intensities in images obtained with time-lapse interference reflection microscopy (IRM). Growth cones from both chick retinal ganglion cells and axons in the GAG treated eyes were found within this novel layer of CS matrix. This repolarization was concentration dependent and was marked by the retraction of the vitreal end feet followed by the relocation of the retinal ganglion cell bodies to the ventricular surface of the neuroepithelium. This newly polarized optic fiber layer was astonishingly indistinguishable from a normal nerve fiber layer except that the individual axons were not oriented in any particular direction.

We show that the ability of immature retinal ganglion cells to initiate axons is not restricted to the vitreal endfoot and that the vitreal endfoot also has this potential. We have also found that cell body and axon polarity in intact retina are caused by the location and concentration of the GAG component of the extracellular milieu.

5.14.10

GROWTH CONES ARE ACTIVELY INFLUENCED BY SUBSTRATE-BOUND ADHESION MOLECULES. S.M. Burden*, H.B. Payne, and V. Lemmon. Dept. of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH, 44106.

As axons advance to appropriate target tissues during development, their growth cones encounter a variety of cell adhesion molecules (CAMs) and extracellular matrix molecules (ECM). Purified CAMs and ECM influence neurite outgrowth in vitro and are thought to have a similar function in vivo. We previously utilized scanning electron microscopy to compare morphological characteristics of retinal ganglion cell (RGC) growth cones on several substrates. We found that growth cone lamellipodial area and number of filopodia per growth cone are affected by the substrate bound adhesion molecule (Payne et al.,1992 Cell Motil. and Cytoskel. 21:65-73). In this report, we use time lapse videomicroscopy to examine dynamic transformations of RGC growth cones as they progress on L1/D9, N-cadherin, or laminin onto a different substrate. Contact made by the leading edge of a growth cone with a new substrate causes a rapid and complete reorganization of the growth cone morphology. Frequently, the changes encompass the entire growth cone including those regions not in direct contact with the new substrate. These studies demonstrate that growth cones are actively influenced by the substrate, probably through a transmembrane signalling mechanism.
The cell adhesion molecule-associated carbohydrate epitope L2/BBK-1 is abundant in peripheral motor nerve, but scarce in sensory nerve (Martini et al. '92). These experiments evaluate the expression of L2 by denervated motor and sensory pathways after reinnervation by motor or sensory axons. Surgeries were performed on the femoral nerves of adult C57B16 mice, in which motor and sensory axons intermingle proximally, but are segregated distally into sensory and motor branches. Six groups were prepared: 1) distal sensory and motor branch repair 2) crossed distal repair, sensory to motor, motor to sensory 3) proximal trunk repair 4) correct intercalated graft-sensory graft in sensory branch, motor graft in motor branch 5) incorrect intercalated graft-sensory in motor, motor in sensory. Axons did not induce L2 expression in sensory or motor pathways. Motor axons induced L2 in some sensory pathways, but correctly reinnervated motor pathways expressed L2 more vigorously. Regenerating axons thus alter pathway characteristics, yet pathways retain evidence of their previous axonal associations. The strong interaction of motor axons with old motor pathways suggests that L2 expression is associated with preferential reinnervation of motor pathways by regenerating motor axons (Brushart '88, '90).

INTERFERENCE REFLECTION MICROSCOPIC (IRM) STUDY OF CHANGES BETWEEN LAMININ AND FIBRONECTIN. T. M. Gomez* and P. C. Letourneau. Departments of Anatomy, and Histology and Neurobiology, Karolinska Institute, S-171 77 Stockholm, Sweden. Growth cones of chick dorsal root ganglia (DRG) neurons exhibit a variety of behaviors in culture at a boundary between glass-adSORBED laminin (LNM) and fibronectin (FN) (Gomez et al., Soc. Neurosci. Abst. 17:738, 1991). Growth cones that encounter LNM while migrating on FN usually cross onto LNM, often with an increased rate of neurite elongation and altered morphology. Reciprocally, growth cones that encounter FN while migrating on LNM orient their direction of migration away from the substratum and maintain association with LNM. It is not known whether differences in substrate adhesion contribute to the behavior of DRG growth cones at LNM-FN boundaries.

To address this question, growth cone contact with LNM and FN treated substrata was assessed using IRM. Our results confirm a previous report that DRG growth cones express closer contacts when growing on FN than on LNM (Gundersen, J. Neurosci. Res. 21:298-306, 1988). A new finding, however, using time-lapse IRM, was that the nature of contacts of a single growth cone changes rapidly as it passes from one substratum onto another, such that a growth cone in contact with both substrata can show two patterns of attachment. Growth cones accelerate and expand their lamelipodia as they migrate onto LNM from FN and simultaneously lose many close contacts, acquiring an IRM pattern typical for migration on LNM alone. Alternatively, growth cones that extend onto FN from LNM increase their total area of close contact and acquire an IRM pattern typical of migration on FN alone.

These data suggest that: 1) the extent of adherence of a growth cone to LNM or FN does not necessitate neurotrophin expression within the CNS. In the ventral funiculus lesioned animals labelled cells were also seen in the motor nucleus neuropil. Low affinity NGF-receptor (LNGFR) mRNA was increased in the scar tissue after both types of lesions, and immunoelectron microscopy localized LNGFR-immunoreactivity mainly to axons and pericellular vessels. Preliminary results regarding localization of mRNAs for the irk-family show all three irks (irk, irkB, and irkC) to be increased in the scar border, neuropil and white matter ipsilateral to the lesion. In addition, both full-length and truncated forms of irkB were also abundantly expressed within the scar tissue. The expression of mRNA for these trophic factors and receptors could be of importance for the permissiveness to axonal sprouting in spinal cord scar tissue. Macrophages which have adhesiveness to the scar tissue, in the absence of blood-brain barrier, may be involved in the regulation of the studied mRNAs.
CHANGES IN EXPRESSION OF JUN AND KROX PROTEINS AND IN NITRIC OXIDE METABOLISM FOLLOWING TRANSSECTION OF THE MEDIAL FOREBRAIN BUNDLE IN THE RAT CNS. T. Herdegen, P. Brecht, F. Bravo* and M. Zillmer; Inst. of Anatomy, University of Regensburg; *Bristol-Myers Squibb Pharm. Incat, Princeton, USA. In the rat, transection of the medial forebrain bundle (MFB) was unilaterally transected by a 1 mm blade via craniotomy in a stereotaxic frame at Bregma -2.0 and expression of JUN, FOS and KROX nuclear proteins was assessed by immunochemistry. This resulted in axotomy of neurons located in the medial thalamus, ncl. mamillothalamicus, ncl. parafascicularis, thalamus, ncl. mammalian, ventral tegmentum and substantia nigra. Neuronal cell nuclei in these areas showed marked and SD rats. Injured and contralateral forebrain bundles (CUN D and KROX-24 (syn. 21f/268, EGR-1, NGFI-A)). The proteins appeared within 12-24 h and had a maximal expression after 48 h. JUN D and KROX-24 declined after 30 days whereas CUN still persisted on a submaximal level for up to 60 days (the longest investigated period). c-FOS, FOS B, JUN B and KROX-20 (syn. EGR-2) proteins were not induced. Further investigations will elucidate dependence of expression on period of survival, age and length of the proximal nerve stumps. MBP leision also induced changes in the nitric-oxide metabolism that are associated with increased activity of nitric-oxide synthase. Supp. by DFG.

CRONIC NERVE LIGATION LEADS TO ENHANCED GABA, RECEPTOR-INDUCED CONDUCTANCES IN A SUBCLASS OF RAT DORSAL ROOT GANGLION NEURONS. D.L. Fink*, G. Richarson, J.D. Keeney, Dept. Neurology, Yale Med. Sch., New Haven, CT. 06510; and VAMC, W. Haven, CT. 06516. Chronic ligation of the rat sciatic nerve elicits both a reduction in primary afferent depolarization and a decrement in GABA-induced depolarization of the L4 and L5 dorsal roots. The aim of the present study was to examine electrophysiological properties of GABA, receptors on dorsal root ganglion (DRG) neurons following chronic axonal injury. Sensitive were disconnected from their targets by ligation and transection. L4 and L5 DRG neurons were cultured in 2.4 wks. Whole cell patch clamp recordings were obtained from 20-42 µm neurons within a day after plating. Patch electrodes (1-3 Mohm) were made from borosilicate glass and filled with 140 mM KCl-based solution. GABA (100 µM) was applied to the entire cell body by rapid pressure microinjection to capture the peak response prior to desensitization. GABA-induced current was measured from voltage clamp experiments using test potentials of -60, -80, -100 mV (>80% series resistance comp.) to calculate conductance changes. In neurons with diameters of 34-42 µm, whole cell conductances of 116±111 pS (n=31) and 446±295 nS (n=26) (p<0.005) were recorded from control and ligated groups, respectively. Muscimol elicited a similar response as GABA, and the responses were blocked by bicuculline. There was no difference in the GABA-mediated conductance change between control and ligated groups in the 20-22 µm neurons. All injured neurons with large GABA conductances had short duration action potentials with no notificable inflections. Control neurons had a variety of action potential types. These data indicate that peripheral nerve ligation leads to a substantial increase in GABA-induced inward current in a subset of DRG neurons (34-42 µm dia). Given that dorsal root axonal GABA-receptor sensitivity is reduced following a similar injury, it is suggested that GABA receptor subtypes may change with chronic injury, but that receptor transport is impaired to the central terminals. Supported by the VA and NIH.

RAPID AND EXTENSIVE GROWTH OF NEURON S ACROSS LESIONS OF IMMATURE MAMMALIAN SPINAL CORD IN CULTURE. J.M. Treherne*, G. Knett, J.G. Nichols and N.R. Saunders; Biocenter, University of Basel, CH-4056 Basel, Switzerland. The isolated central nervous systems of newly born opossums and 15-day rat embryos survive in culture for 7 days or longer. Our earlier work has shown that lesions to the spinal cord in vitro are followed, after 3-5 days, by restoration of conduction and by profuse fiber outgrowth. Experiments have now been made in which cultures were followed with video microscopy the trajectories of axons stained by the biotin and ligated groups, respectively. Muscimol elicited a similar response as GABA, and the responses were blocked by bicuculline. There was no difference in the GABA-mediated conductance change between control and ligated groups in the 20-22 µm neurons. All injured neurons with large GABA conductances had short duration action potentials with no notificable inflections. Control neurons had a variety of action potential types. These data indicate that peripheral nerve ligation leads to a substantial increase in GABA-induced inward current in a subset of DRG neurons (34-42 µm dia). Given that dorsal root axonal GABA-sensitivity is reduced following a similar injury, it is suggested that GABA receptor subtypes may change with chronic injury, but that receptor transport is impaired to the central terminals. Supported by the VA and NIH.

THE INFLUENCE OF SCHWANN CELLS ON EARLY PERIPHERAL NERVE REGENERATION STUDIED BY CHRONICALLY IMPLANTED ELECTRODES IN THE CAT. K. Ferguson*, C. Krum, Dept. of Neurophysiology, Inst. of Med. Physiology, Panum Inst., Univ. of Copenhagen, Copenhagen, Denmark, DK 2200 N. In 21 cats, implanted electrodes with multiple contacts were used to examine the influence of Schwann cells on the rate of elongation after Wallerian degeneration. The right lumbal nerve was crushed and resutured and in the other leg in addition frozen for 20 mm distal to the lesion. The right lumbal nerve was crushed and resutured and in the other leg in addition frozen for 20 mm distal to the lesion. Regeneration was followed by weekly recordings of electrophysiologically evoked responses from axonal sprouts until reinnervation of the plantar muscles occurred after 42-84 days. The action potentials from single regenerating fibers had conduction velocities and amplitudes of 0.5-3 m/s and 0.15-0.5 m V respectively. The spatial relation of the regenerating axons to the stimulation electrodes was examined by electron microscopy showing the presence of axons when an action potential was recordable. The serial electrophysiological observations suggested that the rate of elongation was 3-4 mm/day after crush alone and similar after crush-freezing. However after section and reestablishing a causal injury phase of very slow regeneration suggesting that Schwann cells was critical when the continuity of bands of Büngner had been disrupted. After the initial slow phase, the elongation rate seemed to be independent of freezing.
615.11

The highly specific projection of abducens interneurons (ABD Ints) onto the contralateral medial rectus motoneurons (MR Nms) offers a good model to evaluate the morpho-physiological consequences of target removal in adult CNS neurons. MR Nms were killed by the injection of a cytotoxic into the MR muscle. Following target removal, the overall firing activity of ABD Ints appeared markedly reduced. The neuronal sensitivity to both eye position and velocity showed values significantly lower than controls. ABD Ints also showed a significant reduction in the amplitude of their excitatory and inhibitory synaptic potentials that did not differ from controls. A morphological study demonstrated the absence of cell death in the ABD Ints population, but a progressive decrease in the density of their axonal terminals within their normal area of distribution. Therefore, these adult CNS neurons survive long-term target removal with the maintenance of appropriate physiological signals.

615.12
TOWARDS PULSE PROCESSING NEURAL COMPUTERS FOR BI-DIRECTIONAL COMMUNICATION WITH THE NERVOUS SYSTEM. R. Eckmiller* and M. Janzen. Dept. Biophysica, University of Düsseldorf (FRG)

Monitoring and control of signal time courses of the human nervous system becomes increasingly important for diagnostic and functional repair purposes (e.g. cochlear implants, EKG, FES). The neural interface tissue currently does not allow for an adequate communication with the nervous system as an information processing structure by means of multiple spike trains at the single neuron level.

Neurotechnology is gradually emerging for development of multidisciplinary neural interfaces (MI) and adaptive neuronal computers (ANC) as the two key components for communication with the nervous system by means of asynchronous spike trains via a multi-channel (with 10^6 single neuron contacts) neural interface. This paper reports on the development and test of an ANC prototype consisting of 32 electronic neuron analogs and 64 synaptic analogs with adaptive weights and adaptive delays (Janzen et al. In: 2nd Int. Conf. Microele. of Neural Netw. Munich, Oct. 91). In brief, each of the biology-inspired neuron analogs generates single impulses (1 ms) once its membrane potential (as defined by the asynchronous input of EPSFs and IPSFs from corresponding synapses) reaches the adjustable threshold. In conjunction with a PC to monitor neural parameters in real time and to specify various initial network topologies (via electronic switch arrays), a number of selected applications for pattern recognition, adaptive filtering, and motor control in real time have been successfully tested. With these experiments, some main features of weight- and/or delay adaptation with embedded learning rules (using ‘training neurons’ and presynaptic synapses) were studied. Supported in part by grants from BMFT and MWF.

616.1
IDENTIFICATION AND ANALYSIS OF AN AMYLOID PRECURSOR-LIKE PROTEIN LOCALIZED ON CHROMOSOME 19 W.Wasco. B.T. Hyman and R.E. Tanzi, Dept. of Neurology, MGH, Boston, MA.

We have isolated a cDNA from a mouse brain library that encodes a protein whose predicted amino acid sequence is 42% identical to the amyloid β-protein precursor (APP). This 653 amino acid amyloid precursor-like protein (APLP) is similar to the APP and the Drosophila APLP genes in overall structure and amino acid sequence. The strongest homology occurs in the cysteine-rich, acidic-rich, glycosylated, and cytoplasmic regions. These data suggest that APLP is part of a highly conserved gene family. The APLP cDNA hybridizes to a number of approximately 2.4 and 1.6 kb that are present in mouse brain and neuroblastoma cells. APLP has been mapped to human chromosome 19q13.2-q13.3, the same region on which the APP has been localized. In situ hybridization and immunohistochemical studies show APP and APLP to be expressed in similar human brain regions and cell subpopulations. We are currently testing APLP as a candidate gene for late-onset FAD by standard genetic linkage and mutation analysis. Additionally, we are screening the human genome for other spliced forms of APLP and additional members of the APP gene family.

616.2
TRANSMITTER DEPENDENT INDUCTION OF B-APP mRNA. V. Haroutunian*, S.T. Ahlers*, P.A. Shea*, N. Girenkova, K.L. Davis, and W.C. Wallace. The Mount Sinai School of Medicine, NY and 'N' Neurological Research Institute, Bethesda, MD.

Previous studies have indicated that lesions of the nucleus basalis of Meynert (nBM) induce β-APP synthesis and β-APP mRNA in the ipsilateral cortex. The induction is rapid (within 1 hr) and lasts at least 45 days. We now report on the nature of the stimulus which triggers the β-APP response. Anesthetized rats were implanted with cannulae directed at the nBM and received an infusion of 20% lidocaine. In some of the rats cortical acetylcholine (ACH) release was measured by in vitro microdialysis prior to, and for 90 minutes following lidocaine infusion. These rats were then sacrificed. The remaining rats were re-anesthetized one week later and recovery of ACh release was measured without further lidocaine infusions. Infusion of lidocaine into the nBM led to a rapid reduction (approximately 50%) in cortical ACh release. ACh release had returned to pre-lidocaine infusion levels when measured one week later. Analysis of cortical β-APP mRNA by Northern blot indicated that β-APP was induced immediately after lidocaine infusion. Cortical β-APP mRNA was reduced by approximately 50%, but not one week after lidocaine infusion when cortical ACh release had normalized. These results indicate that β-APP mRNA is reversibly induced when cortical transmitter levels drop below a certain threshold and provide a possible mechanism for β-APP induction in neurodegenerative disease.

616.3
TWO AP-1 SITES ARE NECESSARY FOR THE PROMOTER ACTIVITY OF APP GENE IN ASTROCYTE CELLS. D.K. Lahiri* and C.N. Tang. National Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN-46202

Beta-protein (4.2 kDa molecular weight) is generated by proteolytic cleavage of larger amyloid precursor proteins (APP) encoded by a gene on chromosome 21. The apparent over expression of the APP gene in certain areas of the brain in Alzheimer's disease suggests that abnormal gene expression might be an important factor in the neuropathology. The control of transcription is mediated by different DNA regulatory elements (cis-acting) present in the promoter of the gene. There are about 26 DNA motifs, present in the 5'-flanking region of the APP gene, through which various cell-type specific factors (trans-acting) influence APP mRNA transcription. These data suggest that APP may be part of a highly conserved gene family. The APP promoter was studied by transient transfection assays and mutant promoter constructs. The promoter of the APP gene was analyzed for its ability to direct cell type specific expression. The APP promoter and selected deletions were placed 5' to the reporter gene chloramphenicol acetyl transferase. The promoter deletions were: transcriptionally inactive, different in effect with variant levels of endogenous APP transcripts. Transient transfection assays showed that 96 base pairs 5' to the transcriptional start site are sufficient for full cell type specific promoter activity.

A nuclear factor that binds to a region in a sequence specific manner was identified by mobility shift electrophoresis, DNase footprinting, and methylation interference. The DNase protected domain extends from position -31 to -51 upstream from the transcriptional start site (+1). The NF-κB motif is crucial for factor binding. This sequence overlaps with the consensus sequences for transcription factors AP-1 and AP-4. However, competition experiments suggest that the nuclear factor that binds to the APP promoter is distinct from both AP-1 and AP-4. In addition, factor binding to the characterized recognition sequence is observed in nuclear extracts originating from human, mouse, and rat cells, suggesting a high degree of conservation.

616.4

The major component of amyloid depositions is the amyloid beta protein, which is a truncated 71 amino acid form of the larger amyloid precursor protein (APP). The promoter of the APP gene was analyzed for its ability to direct cell type specific expression. The APP promoter and selected deletions were placed 5' to the reporter gene chloramphenicol acetyl transferase. The promoter deletions were: transcriptionally inactive, different in effect with variant levels of endogenous APP transcripts. Transient transfection assays showed that 96 base pairs 5' to the transcriptional start site are sufficient for full cell type specific promoter activity.

A nuclear factor that binds to a region in a sequence specific manner was identified by mobility shift electrophoresis, DNase footprinting, and methylation interference. The DNase protected domain extends from position -31 to -51 upstream from the transcriptional start site (+1). The NF-κB motif is crucial for factor binding. This sequence overlaps with the consensus sequences for transcription factors AP-1 and AP-4. However, competition experiments suggest that the nuclear factor that binds to the APP promoter is distinct from both AP-1 and AP-4. In addition, factor binding to the characterized recognition sequence is observed in nuclear extracts originating from human, mouse, and rat cells, suggesting a high degree of conservation.

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616.5 REGULATION OF APP SPlicing AND NEUROTYPHIN LEVELS IN THE ADULT RAT HIPPOCAMPUS BY RETINOIC ACID. T. Goddard*, J.B. Pan, L. Montepietra, and S. Watanabe. Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064. It has recently been reported that retinoic acid (RA) is capable of increasing the levels and altering the splicing ratio of APP in cultured SH-SY5Y cells. We have observed a similar effect in these cells. To determine if RA is present in vivo, aged (20-22 month old) male Wistar rats were injected (i.p. at 1 mg/kg body weight, q.d.) with saline, vehicle (DMSO), or low (64 µg/kg body weight) or high (640 µg/kg body weight) doses of RA in DMSO for 2 weeks. The abundance of each of the three major APP species and a control RNA, cyclophilin, were measured by RT-PCR. In the saline injected rats, APP-695 represented approximately 90% of the total APP measured. DMSO treated rats exhibited a 10X increase in total APP (p<0.005) relative to cyclophilin and an increase in the level of APP-695 to 54% of the total APP. Treatment of RA in DMSO decreased the accumulation of total APP relative to cyclophilin at both the low (4X; p<0.01) and high (5X; p<0.05) dosages compared when to DMSO treated rats. Furthermore, the level of APP-695 decreased to 82% with low dosage of RA and 75% at high dosage of the total APP transcripts. In addition we did not detect a significant change in either NGF, NT-3, or BDNF transcripts following low or high dosage RA administration relative to cyclophilin RNA nor did we observe a change in CHAT activity at either of the dosages tested. In conclusion, the effects of RA on APP RNA observed in vivo cells are not indicative of changes in cultured SH-SY5Y.

616.6 A RAT MODEL TO STUDY THE EFFECTS OF BAP-CONTAINING AMYLOID IN BRAIN. A.D. Snow, R. Sekiguchi, D. Nacchi*, K. Kimata, W.A. Schreier and D.G. Morgan. Dept. of Neupath., Univ. of Washington, Seattle, WA 98195; Institute of Molecular Medicine, Aichi Med. Univ., Japan & Div. of Neurogenontology, USC Los Angeles, CA 90089.

Accumulation of amyloid containing beta-amyloid protein (BAP) in the brain is a diagnostic feature of patients with Alzheimer's disease. An animal model is needed to study the formation and subsequent consequences of amyloid deposition in the brain. A rat model provides a rapid in vivo animal model to study amyloid accumulation in brain, and further indicates that a specific co-factor is necessary for amyloid accumulation and its persistence in brain. Support by NIH grants #AG05136, #AG7892, Ad Res.Prog.of the Am. Health Fdn, the French Fdn, for Ad Res., & GliaTech Inc.


We are attempting to generate transgenic mouse models for Alzheimer-type amyloidogenesis based on overexpression of APP protein derivatives in appropriate brain regions. A C-terminal APP-695 segment containing the entire β protein domain (Glue539-Aax695) has been expressed under the control of a chimeric metallothionine-growth hormone promoter system (Stottrup et al, Nature 197:563 [1992]), with sequences replacing the growth hormone coding sequence described in that paper. This transgene is abundantly expressed in transplanted Haela cells, producing novel dense inclusions associated with the endoplasmic reticulum. In extracts of whole brain, Northern analyses reveal that transgene mRNA levels exceed those of the endogenous mRNA up to six-fold. In situ hybridization indicates that the transgene is expressed in pyramidal neurons within cortex and hippocampus. Immunocytochemical analyses with several antibodies to APP epitopes N-terminal to Glue539, reveal elevated intraneuronal accumulation of endogenous APP protein in these brain regions in the transgenic mice. In cortex, this immunostaining is significantly more abundant than in control mice and nontransgenic littermates, with p values ranging from <0.05 to <0.001 depending upon the antibody used. These mice may therefore provide useful models to study the effects of chronic AAP overexpression on brain pathology and amyloid formation.


Three single basepair substitutions in exon 17 of the APP gene have been reported in affected individuals of ten families with familial Alzheimer's disease. All three mutations are predicted to destabilize a stem-loop structure in the APP mRNA which occurs in the 3' end of the sequence encoding the amyloidogenic βA domain (nucleotides 1906 and 1954; APP695 sequence). The stem loop resembles iron-responsive elements (IREs) present in the 5' and 3' untranslated regions of the mRNAs for ferritin and transferrin receptor, respectively. The stem-loops in these messages control translation in response to iron concentration via RNA-binding proteins. We have shown that the IRE-like stem-loop in APP messages binds a novel human brain RNA-binding protein. Preliminary studies indicate that this binding is abolished when the APP177(1-5) is introduced into the stem. We are also testing the effects of the other two mutations on the RNA-protein interaction. Data will be presented on our attempts to isolate and clone the APP RNA-binding protein. Additionally, the effects of the Alzheimer disease mutations on APP mRNA stability and translation are being assessed.


The secreted form of the amyloid β44-protein precursor (APP) is involved in the growth regulation of fibroblasts (Saitho et al., Cell 58:615-622, 1989). Recently, we have shown that the region of the secreted form of APP-695 necessary for this growth regulation is contained within a 40 amino acid domain which is adjacent and C-terminal to the KPI insertion site of APP-751. This active site begins at Thr296 and extends to Met335 (Roch et al., J. Biol. Chem. 267:2214-2221, 1992). To define more precisely the site of activity, we synthesized several peptides spanning the 40 amino acid domain. In addition, we also prepared bacteria-maded APP-751 (secreted form), as well as mutant forms of APP-695 and -751 (secreted forms). These deletion mutants were lacking the majority of the 40 amino acid domain. Each of the peptides, as well as the bacteria-made APP variants were tested in our biological assay on A-1 fibroblasts (a cell line that produces very low APP levels and is dependent on exogenous APP in the medium for normal growth). Our results narrow down the active region of APP-695 from the domain of 40 amino acids to a region of 17 residues starting at Ala319 and extending to Met335.
BIOLOGICAL ACTIVITY OF THE AMYLOID 8/4 PROTEIN PRECURSOR: II. NEUROTOXIC EFFECT OF AN APP PEPTIDE ON A RAT BRAIN NEUROBLASTOMA CELL LINE.


We previously demonstrated that secreted forms of amyloid 8/4 protein precursor (APP) promote growth of fibroblasts (Cell, 52:615, 1989), and adhesion of PC12 cells to substrate (Neuron, 2:699, 1989). The growth promoting activity of APP is fully retained in a synthetic 17-mer peptide corresponding to Ala319-Met335 of APP-695 (Roch et al., reported in this meeting). In order to investigate the effect of the 17-mer APP peptide on neuroblastoma cells, we used a rat B103, a neural cell line derived from rat brain that does not express APP. The neuronal phenotype of B103 has been documented (Nature, 242:224, 1974). We found that the rat CNS neuroblastoma cultures treated with 17-mer APP peptide for 20 hours had longer neurites and more neurite-bearing processes. The significant effect was seen at 10 nM to 200 nM, with maximal effect at 100 nM. The peptide with sequence reverse to that of 17-mer did not have any effect at the same concentration range. This result demonstrates that a small stretch of sequence in the secreted form of APP-695 has both growth promoting and neurotoxic activities.

DEGENERATIVE DISEASE: ALZHEIMER'S — FRIDAY A M

D.V. Pow, D. K. Crook, I.C. Gynther,* and D.I. Vaney. Vision, Touch and glycine, glutamate, and GABA. These antibodies are used routinely for the diagnosis and detection of small molecules including the transmitters of the right side of the brain.

We have devised a simple new technique which permits the rapid identification of extremely high titres of polyclonal antibodies against a wide variety of small molecules including the transmitters glycine, glutamate, and GABA. These antibodies are used routinely for the diagnosis and detection of small molecules including the transmitters of the right side of the brain.

This study demonstrates that melanin receptor binding sites are widely distributed in the forebrain and midbrain of the lizard, as well as in the retinas of the lizard Anolis carolinensis using in vitro autoradiography and computer-generated color imaging. Radiolabeled labelling was observed in areas which receive primary, secondary, and tertiary visual input; the superficial layers of the optic tectum, lateral geniculate nucleus, nucleus rotundus, dorsal ventricular ridge, striatum, and interpeduncular nucleus. Other areas that demonstrated binding included the medial habenular nucleus, medial cortex, dorsal cortex, mammillary nucleus, and septum. In the retina, melanin binding was localized in the inner plexiform layer. An asymmetry of melanin binding was seen in the diencephalon: a high degree of melanin binding was present in the left medial habenular nucleus, and no binding was observed in the habenulum on the right side of the brain. This study demonstrates that melanin receptor binding sites are widely distributed in the forebrain and midbrain of the lizard, and that these observations suggest the that left habenulum is under dual control (neuronal and hormonal) of the parietal eye/pinna. complex, and that melanin may play a significant role in neural processing of visual information.


617.3 STRATEGIES FOR THE PRODUCTION OF EXTREMELY HIGH TITRE ANTISERA AGAINST SMALL NEUROTRANSMITTER MOLECULES. D.V. Pow, D.K. Crook, I.C. Gynther* and D.I. Vaney. Vision, Touch and Hearing Research Centre, University of Queensland, Brisbane 4072, Australia.

We have devised a simple new technique which permits the rapid identification of extremely high titres of polyclonal antibodies against a wide variety of small molecules including the transmitters glycine, glutamate, and GABA. These antibodies are used routinely for the diagnosis and detection of small molecules including the transmitters of the right side of the brain.

This study demonstrates that melanin receptor binding sites are widely distributed in the forebrain and midbrain of the lizard, as well as in the retinas of the lizard Anolis carolinensis using in vitro autoradiography and computer-generated color imaging. Radiolabeled labelling was observed in areas which receive primary, secondary, and tertiary visual input; the superficial layers of the optic tectum, lateral geniculate nucleus, nucleus rotundus, dorsal ventricular ridge, striatum, and interpeduncular nucleus. Other areas that demonstrated binding included the medial habenular nucleus, medial cortex, dorsal cortex, mammillary nucleus, and septum. In the retina, melanin binding was localized in the inner plexiform layer. An asymmetry of melanin binding was seen in the diencephalon: a high degree of melanin binding was present in the left medial habenular nucleus, and no binding was observed in the habenulum on the right side of the brain. This study demonstrates that melanin receptor binding sites are widely distributed in the forebrain and midbrain of the lizard, and that these observations suggest the that left habenulum is under dual control (neuronal and hormonal) of the parietal eye/pinna. complex, and that melanin may play a significant role in neural processing of visual information.


In the brain, glutamate receptor subunits are distributed throughout the cerebral cortex, cerebellum, and hippocampus, among other brain regions. The lack of specific immunohistochemistry for any of these glutamate receptor subunits has hampered our understanding of the functional role of these receptors in the brain. We have generated monoclonal antibodies against the kainate (K) class (GluR5-GluR7) glutamate receptor subunits. Analysis of the distribution of the K class subunits in the temporal cortex of the macaque reveals that the K class subunits are distributed in a highly specific and selective manner.

In the entorhinal cortex, D- and D1A peptide antiserum to K subunits revealed specific labelling of the hippocampus and entorhinal cortex. Antibodies to the K class subunits label the soma and dendrites of pyramidal neurons within all CA fields and subiculum, as well as the dendrites of the granule cells of the hippocampus. The soma of the granule cells are conspicuous by their absence of immunoreactivity for the K subunits. In the entorhinal cortex, the soma and spiny dendrites of neurons within layers V and VI extend to superficial layers were prominently labeled while only the somas and sparse proximal dendrites of layer II and III cells were labeled. This dense pattern of labelling was also present in the perirhinal region while the predominantly unimodal visual region of the inferior temporal cortex was sparsely labeled. The polymodal region within the superior temporal sulcus was heavily labelled as was the insula.

Neurons of layer V of the entorhinal cortex identified by retrograde labelling with fast blue as projecting to STS was filled with lucifer yellow. Double labelling with antibodies to the K class subunits demonstrated these neurons to be K immunoreactive subunits. Immunoreactivity is present in the soma and primary dendritic shaft of these neurons but is excluded from distal dendrites. Supported by NIH grant AG06647 and American Health Assistance Foundation (AHAF).
617.5 DISTRIBUTION OF GLUTAMATE RECEPTOR SUBUNIT PROTEINS IN MONKEY NEOCORTEX. L.J. Morrison*, J.C. Vickers, G. Huntleyl, T.E. Goodl, W.G. Janssen1, N. Archin1, T. Moran2, S.W. Rogers3, and S.F. Heinemann4:

- We recently developed monoclonal antibodies (MAb) to several of these subunits, and with a brain:blood ratio of 5:1 and 0.6% injected dose/gr brain at 5 min. Following the tail vein injection of 100gCi [3H]apam etol (60 Ci/mmole), the ligand readily crossed the blood-brain-barrier and accumulated in the rat brain, with a brain:blood ratio of 5.1 and 0.6% injected dousiugl brain at 5 min. The distribution of activity at 1 h following injection was similar to the distribution of ν2 adrenoceptors observed in vitro. Coinjection of Idoaxan 2mg/kg produced a significant reduction in brain activity in vivo. Thus, appropriately labeled atipamezol shows promise as an imaging agent for ρ2 adrenoceptors with high resolution positron emission tomographic PET.

617.6 DETECTION OF ν1-NEUROPEPTIDE Y BINDING SITES IN RAT BASILAR ARTERY BY QUANTITATIVE AUTORADIOGRAPHY OF THE DOPAMINE D3 RECEPTOR IN HUMAN BRAIN. C. Schmauss, H. Harronan and J.A. McQuade*. Dep't Psychiatry, Mount Sinai School of Medicine, New York, NY 10029.

- The feasibility of using [3H]apam etol as an in vivo and in vivo imaging agent for brain ν2 adrenoceptors was examined with ν2 autoradiography in the rat and in vitro autoradiography in rat and human brain postmortem. In vivo autoradiography was performed by applying 0.5nmol radioligand to 20g rat or 40g human brain sections for 1 hour at room temperature, followed by 2 X 10 min wash in ice cold incubation buffer. Nonspecific binding was determined in the presence of 10μM unlabeled clonidine. Sections were dried and apposed to tritium sensitive film for 4 weeks. A computerized image analysis system was employed to measure standards and regions of interest and to compute regional specific binding. Distribution of specific [3H]apam etol binding in rat and human brain was compatible with the known distribution of ν2 adrenoceptors as demonstrated by in vivo autoradiographic studies using agonists and the antagonist idazoxan but not rauwolscine. Following the tail vein injection of 100gCi [3H]apam etol (60 Ci/mmole), the ligand readily crossed the blood-brain-barrier and accumulated in the rat brain, with a brain:blood ratio of 5.1 and 0.6% injected dousiugl brain at 5 min. The distribution of activity at 1 h following injection was similar to the distribution of ν2 adrenoceptors observed in vitro. Coinjection of Idoaxan 2mg/kg produced a significant reduction in brain activity in vivo. Thus, appropriately labeled atipamezol shows promise as an imaging agent for ρ2 adrenoceptors with high resolution positron emission tomographic PET.
**618.1**

**NEURITE OUTGROWTH IN PRIMARY CULTURES OF DROSOPHILA PHOTO-RECEPTOR AND OPTIC LOBE CELLS.** Chinglu Li and I.A. Meinertzhagen. Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The visual system of Drosophila melanogaster has been widely used in developmental neurobiology, for which successful in vivo cultures of eye imaginal discs and optic lobe neurons offer a powerful prospective tool. We have established a primary culture system, with which, for the first time, we have obtained constant neuronal differentiation from both optic lobe and eye imaginal discs. Late third instar larval or white prepupal wild-type Drosophila melanogaster raised at 29°C were surface sterilized, and the eye imaginal discs and the lateral poles of the supranephrogenal hemispheres (containing the optic lobe) dissected out under sterile conditions. Eye discs were partially dissociated by mechanical trituration, while optic lobe cells were dissociated by trituration after 10min in 0.25% trypsin. Preparations were cultured in Nunc dishes coated with poly-L-lysine at 25°C in a humid chamber using the bicarbonate-free Leibovitz L-15 medium supplemented with 10% fetal bovine serum. Neurite outgrowth was observed in both cultures within a few hours of initiating the culture. The cells are immunoreactive to anti-HRP, which recognizes an epitope on insect neurons. Optic lobe cells differentiated as single cells with bifurcated fibers, or as clusters with neurites that fasciculate together. Occasionally ganglion cells about 5µm in diameter clustered around a larger round cell (diameter ~10µm), presumed to be their stem cell neuroblast. Some processes from these neurons had clear growth cones and varicosities. Cultures have been maintained up to 10d. For successful culture of eye imaginal discs it is important to dissociate the discs only partially. Disc fragments usually remain inside vesicles of various sizes. Healthy neurite outgrowth is usually observed from these vesicles or clusters, although single cells with long fibers are also seen; growth cones can be seen in some cells. Anti-HRP antibodies indicate that neuronal differentiation occurs in these eye disc fragments, which also give rise to non-neuronal cells.

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**618.2**

**ELECTRICAL PROPERTIES OF PEPTIDERIC NEURONS ARE UNCHANGED WITH TIME IN CULTURE.** D.E.R. Meyers and L.M. Cook. Belfsky Lab. of Neurobiol., Univ. of Hawaii, Honolulu, HI 96822, USA.

After 1 d in defined culture, regenerating crustacean (Cardioma Carcinus) peptidergic neurons show veiling or branching morphologies. Peptidergic neurons from a defined medium show immediate outgrowth with characteristic patterns including veiling (associated with immunoreactivity to crustacean hyperglycemic hormone antisera) and various types of branching (Cook et al., 1989; PNAS 86:402). We have now reviewed our previous findings and larger branching neurons after 5-6 d in culture. Using whole-cell patch recording (pipette, 300 MΩ K⁺, bath, 2 ml saline), 3 of 4 veiling but only 1 of 5 branching cells fired overshoot spikes. Under VC, all 6 veiling cells tested showed inward (0.3-1.9 nA) and outward (1.1-1.3-3.9 nA, Vc 0 mV, Vh -40 mV) current, while only 3 of 12 branching cells did (<0.5 nA). The mean soma diam. of these cells (44±6, K+ 145 ±30, Vc 0 mV) was significantly larger (1)(17±6, P = .004) than that of the others (25±6 µm). Max. outward current at 0 mV (Vc -40 mV) in the 12 cells was 0.6-3.5 nA. Sixteen branching cells were examined in solutions designed to isolate L type. Genenerated (max. 134±32 µA) pA). The mean soma diam. of these cells (37±9 µm) was significantly larger (1)(11.5, P = .02) than that of cells devoid of L (25±7 µm). We conclude that the electrical properties of the cells previously studied at 24 h do not change substantially over the following 5 d. The large-diameter, branching cells which generate I, were not targeted in our earlier study as they could not be distinguished from veiling cells that had not produced a lamellipodium by 24 h. Supported by NSF BNS-8910432 and the Univ. of Hawaii Foundation.

**618.3**

**CALCIUM TRANSIENTS IN RESPONSE TO CONDITIONING FACTORS IN REGENERATING HELIOLOMA NEURONS.** D.K. Kane* and C.S. Cohon. Dept. of Anatomical Sciences, SUNY at Buffalo, Buffalo, N.Y. 14214.

Previous studies have shown a correlation between neurite elongation and intracellular calcium levels in Heliconia neurons (Cohon and Kater., 1987). This study examined whether the initiation of outgrowth from a cut axon stump was associated with changes in intracellular calcium levels in response to the presence of conditioning factors.

Identification of the Heliconia neuron, B19, was removed from the buccal ganglia with an attached piece of axon and plated into defined culture medium. Cells were injected with Fura-2 and calcium levels were monitored at the distal axon before and at various times after the addition of conditioning factors. In the absence of conditioning factors, no significant changes in calcium levels were observed and calcium levels remained constant. However, the addition of conditioning factors resulted in the extension of new neurites from the distal axon together with cyclical elevations in intracellular calcium that were first detected at 8-12 hours. Approximately 50% of the cells exhibited calcium transients. These transient elevations had peak amplitudes that were 20-30% above basal calcium levels and a period of about 10 minutes. Transients were blocked by lanthanum indicating that the calcium originated from the plasma membrane. Blocking calcium transients with lanthanum did not inhibit the initiation of outgrowth. However, the rate and extent of outgrowth was significantly lower.

These calcium transients and the associated stimulus factors to Heliconia neurons induces calcium transients. While the calcium transients do not appear to be directly related to the initiation of outgrowth, they do appear to have some effect on the rate and extent of outgrowth.

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**618.4**


There are conflicting observations on the relations between [CA²⁺] in neurons and the outgrowth from cultured neurons. This report provides observations pointing to the capability for CA²⁺ homeostasis as an important attribute of neurons showing outgrowth. Peptidergic neurons from the X-organ - sinus gland sensorimotor system of land crabs (Cardioma gastrodermis) from a defined medium show immediate outgrowth having several characteristic patterns including veiling (associated with immunoreactivity to crustacean hyperglycemic hormone antisera) and various types of branching (Cook et al., 1989; PNAS 86:402). A few neurons show little regeneration; as we compare the responses of cells lacking outgrowth with extensively-regenerated cells (from same culture) after application of depolarizing [K⁺, 15X normal; 1-7 min]. [CA²⁺] was detected by FMRFamide using the ratiometric equation with Rm in = 0.4, Rm max = 6.0, and Kd=800 nM. In both morphological classes, basal [CA²⁺] ranged from 50-200 nM and increased at least 200 nM in response to [K⁺]. In processes as well as somata, time courses of [CA²⁺] in neurons it remained high. New [CA²⁺] steady state levels were established within 20 min. The cell's ability to regulate perturbations of [CA²⁺] may be critical to neuronal outgrowth.

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**618.5**


The aim of our experiments is to investigate how substrate molecules of the extracellular matrix (ECM) influence the growth and regeneration of nerve cells. Earlier work has shown that the growth of leech neurons in culture depends on substrate molecules, each substrate producing a characteristic pattern of neurite outgrowth. In particular, a laminin-like molecule induces slender, straight and relatively unbranched processes. In vivo, laminin appears in the regenerating CNS closely associated with regenerating fibers. One question is whether the laminin is newly synthesized or displaced from other sites. Metabolic labeling of regenerating CNS in culture with 35S-methionine indicates that laminin synthesis increases one week after amputation. Tests have been made to identify the cellular components that make the laminin. Two findings implicate microglial cells: (1)ry accumulation of laminin correlates with the migration of microglial cells to the cut end of microglial cells in culture show immunoreactivity to laminin by immunofluorescence staining. To clarify further the association of laminin with microglial cells immunolabeling is being status may be used to control genetically. In parallel, FMRFamide reduces significantly the frequency of fasciculation by L7 growth cones on other sensory cells. As reported for 5-HT effects on sensory cells, the down-regulation of apCAM on L7 by FMRFamide may involve its internalization. Cell-specific changes in the pattern of a group of neuron-specific membrane glycoproteins in the motor cell L7, is down-regulated by applications of 5-HT that evoke long-term facilitation or inhibition of the sensorimotor synapse evoked by 5-HT or FMRFamide, respectively.
618.7 EVIDENCE FOR A ROLE FOR TYROSINE PHOSPHORYLATION IN REGULATING THE PERIPHERAL ACTIN NETWORK OF THE GROWTH CONE. D.Y. Wu and D.J. Goldberg, Centre for Neurobiology and Behavior, Columbia University College of Physicians and Surgeons, New York, NY 10032

The network of actin filaments in the periphery of the growth cone is specialized in its organization and activities and plays a critical role in the formation and movement of protrusive structures (filopodia, veils and lamellipodia). We have identified an Mr 618.9 kDa Fabs block growth cone initiation, or cause collapse and receptor protein-tyrosine kinases apparently rapidly and locally initiates the development of peripheral actin systems even in the absence of growth factor. Immunofluorescence microscopy with a monoclonal antibody to phosphorytoxine reveals staining throughout the growth cone but especially bright staining at the tips of most filopodia. Treatment with an inhibitor of protein-tyrosine kinase (PTK), genistein, eliminates the tip staining from most filopodia. VEC-DIC microscopy reveals that genistein and another PTK inhibitor, lavendustin A, rapidly cause a temporary elongation of filopodia as well as a disappearance of the (hundreds of actin filaments) in lamellipodia. In addition, either inhibitor completely prevents the formation of filopodia and veils that normally occurs neurally along an Aplysia axon when it is transected in culture. These results suggest that protein tyrosine phosphorylation, even in the absence of growth factors, is important in regulating the peripheral actin network of the growth cone. One important site of interaction of tyrosine phosphorylated proteins and the actin network may be the tips of filopodia.

618.8 DEVELOPMENTAL EXPRESSION OF G PROTEINS IN MIGRATORY NEURONS OF THE INSECT ENTERIC NERVOUS SYSTEM. P.F. Copenhaver* and R. Taken in the developing enteric nervous system (ENS) in the mouse, Manduca sexta, involves the migration of about 400 neurons, the EP cells, along a series of pre-formed pathways on the gut musculature. We are using this system to investigate the regulation of G protein expression, duration, and directionality of the cell migratory process. Specifically, we have examined the developmental expression and possible function of the heterotrimeric guanylate cyclase-binding protein (G proteins) in the course of neuronal migration. Antibodies against the α-subunits of G proteins cloned from ENS neurons (Veenstra and colleagues, 1987, 1988) were used to map the patterns of G protein expression during embryonic development. While none of the G proteins could be detected in the migratory EP cells, all of the cells began to express detectable levels of Gα at the time of migration. The intensity of staining gradually increased during the migratory period, with immunoreactive material distributed throughout the axonal processes of the EP cells as well as their somata. When preparations were exposed to 10 μM aluminum fluoride for 30 min just prior to the onset of migration, we found that cell migration was completely blocked. In addition, we have identified an Mr 618.11 kDa protein that is present in migratory EP neurons and is blocked by treatments that block actin-based filopodial extension in part by extension of filopodia within neuropils; it can be blocked by treatments that block actin-based filopodial extension independently, we plan to interfere with neurite outgrowth-promoting molecules in Octopus nervous system by using Fabs and examine the effect on neurite outgrowth. We have evidence that tyrosine phosphorylation in Octopus is mediated in part by extension of filopodia within neurotubes; it can be blocked by tyrosine phosphorylation blocking rhodamine-labeled phalloidin (250μg/ml TCA) into Ti 1 cell bodies and allowing it to diffuse throughout the cell. Dense F-actin labeling was observed in filopodia and small branches that extended small branches that extended from the growth cone, and in the peripheral cortical actin network of the axon; little staining was observed in the residual volume of the axon the growth cone. Within approximately 2-4 hours of the addition of cytochalasin D (10 μg/ml), phalloidin labeled F-actin collapsed. If cytochalasin was washed out within 30 min. of initial application, the original pattern was rapidly reconstituted. Following application of cytochalasin to DIO labeled growth cones, some (presumably short) filopodia retract into the growth cone. However, in every growth cone treated many lengthy filopodia remained extended for periods up to two hours (after 10-20 they eventually were withdrawn). Video imaging of DIO labeled growth cones double-labeled with phalloidin confirmed that F-actin did depolymerize following cytochalasin application. These results suggest that individual filopodia form periodic substrate attachments; these attachments may maintain filopodia for several hours in the absence of Factin. If this is the case, it may account for both the unusual length of filopodia in situ, and the ability of individual filopodia to steer along favorable guidance substrates.

618.10 VIDEO MICROSCOPY OF VACUOLES WHICH FORM FOLLOWING OSMOTIC PERTURBATION OF MOLLUSCAN GROWTH CONES. L.A. Harris, K.E. Smith* and C.E. Morris, Labo Institute (and Biology Dept.) University of Ottawa, Ottawa, Ontario, Canada, K1N 6G9

Molluscan neurons regenerating in culture (usually unidentified Lymnaea neurons, occasionally Aplysia bag cells) were subjected to a variety of acute (minutes) and longer term (hours) osmotic shocks. Cells were monitored on video. Within minutes of return to isosmotic medium, vacuoles (previously hypoosmotic vacuoles or pHOSvacs) formed in the growth cones and at adhesion sites; surprisingly, they disappeared within minutes when neurons were re-stimulated. The process was repeatable and the pHOSvacs charcteristics reappeared in their previous locations. Isolated growth cones were competent to form pHOSvacs. pHOSvac formation did not represent irreversible damage to the neurons. Time lapse video of neurons exposed to a distilled water pulse (<2 min) followed by return to isosmotic solution (inducing pHOSvacs) demonstrated that over 24 h most of the pHOSvacs disappeared and neuronal arborization processes (e.g. lamellipodial searching, axonal transport, retrograde ruffling) continued. Both pHOSvac formation and their rapid disappearance during a second HOS seem sensori-twin; pursuing the phenomenon we hope to learn something about processing of cytoplasmic lipid pools. Supported by NSERC, Canada.
618.13


Tonically and phasically active crayfish axons and motor terminals have well-characterized differences in morphology (Lnenicka, N. Y. Acad. Sci. 627:197-211, 1991). To investigate the ontogeny of these differences, we have developed a culture system in which we can observe the regenerative growth of crayfish tonic and phasic motor axons. Abdominal nerve cords, including ganglia one through five, were dissected from juvenile crayfish and plated onto coverslips in culture medium. Within three days, growth was observed from the cut ends of the phasic and tonic branches of the third root. Using standard electrophysiological techniques, we determined that tonic and phasic neurons in culture for one week, retain their different electrical activity patterns. Many tonic cells were spontaneously active, while phasic cells showed no spontaneous impulse activity but responded to stimulation of their cell bodies.

In our initial studies, we examined the growth patterns of the tonic and phasic motor axons. Cultures were photographed, and the negatives projected and traced. Either length or area of growth was calculated and branching patterns described. Axonal growth was observed as early as 24 hrs after plating and continued for an additional 7 to 10 days. Three patterns of growth repeatedly occurred within a single culture dish: 1) slender with little or no branching, 2) bushy with extensive branching, 3) extremely wide and flat with little branching. Growth rates and branching pattern of axons differed between the two populations of cells. Growth from tonic axons was almost exclusively type 1, while the growth of the phasic cells consisted of all three types. (Supported by NSF grant BNS-9121757)

618.14

COLCHICINE BLOCKS AXONAL REGENERATION IN AN IDENTIFIED NEURON OF HELISOMA. P. J. Krusk* and A. G. M. Balloch. Dep. of Medical Physiology, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Colchicine is known to bind to microtubules and block axonal transport in many animal classes, including mollusks and gastropods, it also blocks neurite elongation in mouse neoblastoma and PC12 cells. We tested the effect of colchicine on adult Helisoma buccal neuron 4 (B4) regenerating after axotomy. This neuron has two axons, one in the ipsilateral and one in the contralateral esophageal nerve trunks, which together innervate the paired salivary glands. The experiments were performed on semi-intact preparations that consisted of the buccal mass, the salivary glands, and all the central nerves, including the buccal ganglion. Axotomy was performed by crushing both esophageal nerve trunks at a distance of 200-400 µm from the buccal ganglia (20-40% of the nerve length). These semi-intact preparations were cultured separately in medium with various concentrations of colchicine. After culturing for 6-7 days, one of the two neurons B4 in each semi-intact preparation was injected with Lucifer yellow to test its morphology. In control experiments (no colchicine in medium), both axons of the neurons B4 showed extensive regenerative neurite outgrowth. However, neurite outgrowth was blocked in the presence of micromolar or millimolar concentrations of colchicine. It is concluded that colchicine prevents axonal regeneration in Helisoma neuron B4, presumably by blocking axonal transport.

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618.15


The bilaterally symmetric, serotoninergic mesencephalic giant (MCG) cell projects to the buccal ganglia of Achatina fulica via the ipsilateral cerebrobuccal connective (CBC). This innervation is predominately unilateral providing only the 5-HT in the buccal ganglia. Serotonin-like immunoreactivity (SLIR) in the buccal ganglia 3-7 days after a cut to the CBC, reveals a unilateral loss of 5-HT immunoreactivity in the lesioned ganglion, suggesting the degeneration of severed fibers from the MCG. While ipsilateral regeneration is prevented by a cut to the CBC, SLIR nevertheless returns to the lesioned ganglion 3-4 wks after the lesion.

Dye fills and electrophysiological measures reveal that this serotoninergic innervation is the result of neuritic sprouting from the contralateral, uninjured MCG (cMCG) into the denervated ganglion. We have previously shown that lesions of the DG in the EC lesions model are unlikely to be of functional cholinergic nature. The methylcarbamylcholine (20nM)/nicotinic binding sites are significantly decreased in the ipsilateral molecular and granular layers of the DG from 8 to 20 DPL. As growth rates and branching patterns of axons differed between the two populations of cells. Growth from tonic axons was almost exclusively type 1, while the growth of the phasic cells consisted of all three types. (Supported by NSF grant BNS-9121757)

619.1

NICOTINIC ACETYLCHOLINE RECEPTORS ARE DECREASED IN RAT DENTATE GYRUS FOLLOWING ENTRORHINAL CORTEX LESIONS. L. Albert*, J. Fricker, A. Brechard, S. Guastier and R. Guastier. Douglas Hospital Research Center, Deps. of Psychiatry and Neurology & Neuroscience, Center for Studies in Aging, McGill University, Montreal, Quebec, Canada, H3G 1R3.

It has been shown that lesions of the entorhinal cortex (EC) in rat depressed the spontaneous release of acetylcholine (AChE) positive fibers in the dentate gyrus (DG) of the hippocampus. It was suggested that these fibers were cholinergic in nature and originate from the septum nucleus. In our model, lesions of the EC in adult Fisher-344 rats were performed and the status of various cholineric markers was assessed using receptor autoradiography at various days post-lesions (DPL) (2, 4, 8, 14, and 30). As reported by others, (AChE) staining was significantly increased in the ipsilateral DG from 8 to 30 DPL. In contrast, [3H]H4-2133 (25nM)/nicotinic in the septum nucleus (SEN) was decreased in the ipsilateral DG from 8 to 30 DPL. Taken together these findings reveal that spreading AChE-positive fibers of the EC lesions model are unlikely to be of functional cholinergic nature. The decrease in nicotinic binding sites further support this contention on the basis of their likely presynaptic localization in the septo-hippocampal pathway. Supported by the Alzheimer Society of Canada, the American Health Assistance Foundation and MRCC.

619.2

NICOTINIC ACETYLCHOLINE RECEPTOR ALPHA-3 SUBUNIT mRNA IN RAT VISUAL CORTEX: ONTOGENY AND EFFECTS OF NEONATAL ENUCLEATION. T.A. Austin*, S.M. Grady and J.L. Fuchs. Dept. Biological Sciences, University of North Texas, Denton, TX 76203.

The present study examines the role of the alpha-3 nicotinic AChR subunit in developing rat visual cortex. Brain sections from hooded rats were used for in situ hybridization. Slide-mounted sections hybridized with antisense or sense (control) mRNA were exposed to film and were used for the semiquantitative analysis in autoradiographs from P0-P2 rats, alpha-3 mRNA was not yet evident in the visual cortex, but by P5, dense label in layer IV distinguished area 17 from other neocortical areas. During the second postnatal week, alpha-3 levels increased in layer IV throughout the neocortex, but remained highest in area 17. The developmental time course resembles that of geniculocortical innervation, suggesting that alpha-3 mRNA was not yet evident in the visual cortex, but by P5, dense label in layer IV distinguished area 17 from other neocortical areas. During the second postnatal week, alpha-3 levels increased in area 17. The developmental time course resembles that of geniculocortical innervation, suggesting that alpha-3 mRNA expression is associated with postsynaptic targets for neuronal sprouting. This possibility was tested by administering exogenous 5-HT in conjunction with a cut to the CBC. Sprouting from the MCG after injection of 5-HT (0.5 mg every second day) was considerably retarded when compared with the normal sprouting response seen 3-4 wks following lesion with vehicle control, DA and 5-HTP injections. These results support a previously suggested role for 5-HT as a neurite modulator in the central nervous system of the snail.

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ACetylcholine receptor expression in Long term primary cultures of Mammalian myotubes: C.G. Carpenter, A.M. Bode, M.J. Blake, Y. Feng, J. Faber, B.I. Milavetz. Dept. of Physiology, Pharmacology and Toxicological Medicine, UCLA. School of Medicine, Los Angeles, CA 90095-1789.

In order to examine synaptic-independent mechanisms for regulating the expression of the embryonic (E) and adult acetylcholine receptor (AChR) subunits, a protocol has been developed for preparing and maintaining long term primary cultures of mammalian myotubes. Single channel studies of AChR activity from 9 culture runs revealed both E-AChR (expression of mammalian embryonic (E-) and adult acetylcholine receptors) and A-AChR (expression of mammalian adult (A-) AChR subunits) at a common pipette reversal potential of 67.7 ± 1.8 mV (N=55). The ratio of E-AChR to A-AChR varies depending on the event classes (E-AChR:A-AChR) was 0.53 ± 0.03 (N=21). E-AChR accumulated to maximum levels (maximum channel/batch) between culture days (CD) 10 and then declined (by about 50%) to a minimum between CD 25 and 29. Northern blot determinations showed a corresponding reduction in E-AChR mRNA levels between early (CD10) and late (CD21) culture periods. Evidence for synaptic-independent AChR expression is based on patch clamp data showing developmental increases in A-AChR activity. Although this expression was lower and more variable than that at intact endplates, up to 40% A-AChR events were observed in individual patches over a 3 day period of expression. The relationship between this expression and that of motoneuronal development is under investigation.

We thank J.P. Merlie and P. Gardner for kindly providing AChR subunit cDNAs; NSF-EPSCOR 90.


In adult Aplysia, identified serotonergic (5-HT) neurons in the cerebral ganglion (CG) contribute importantly to synaptic plasticity (Mackey et al., 1989). Some of these 5HT cells have been identified in embryonic and larval Aplysia using whole-mount immunocytochemistry (Morais & Carey, 1990). As an initial analysis of the development of these cells through the period of development during the nervous system, we have examined the fine structure and projections of the 5-HT neurons of the CG using immunocytochemistry at the LM and EM level. Our results confirmed the presence and fate of 5 serotonin-like immunoreactive (5-LIR) cells in the CG at the onset of the larval period: two bilateral pairs of cells apparently belonging to the ACC cluster of adult Aplysia (Nolen & Carey, 1986) and a median unpaired cell that is lost at metamorphosis. There are no 5-LIR projection trajectories in the project centrally to a dense neuritic plexus in the CG, where large SLIR fibers associate with non-serotonergic neurons. 2) One of the bilateral pairs of cells and the unpaired median cell send forward projections to the velum, where they appear to contact both myocytes and velar ciliated cells, supporting a role for 5-HT in ciliary beating. 3) SLIR fibers also seem to interact with myocytes of the body wall and internal muscle bundles. 4) Another SLIR fiber, apparently from the second bilateral pair of cells, appears to follow the pleuroabdominal tract of the CNS. This fiber extends posteriorly and ventrally from the CG through an ensheathed nerve bundle to a cluster of cells located dorsal to the statocysts. It then joins a neuropil, and then continues more caudally at the lateral-ventral margin of the esophagus. The course of this fiber, together with the known ontogenetic position of the other central ganglia, suggests that it travels through the anlage of the pleural and possibly the abdominal ganglia. Thus this behavior will be of interest to determine the anatomical connections and possible functional roles of this identified serotonergic projection.


MDMA has been demonstrated to be a potent and selective neurotoxicant for specific serotonergic neurotransmitter systems. Persistent depletions in serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels as well as long-term loss of 5-HT uptake sites and tryptophan hydroxylase activity are observed following administration of MDMA to experimental animals. Sprague Dawley rats, males and females, were given a single intraperitoneal dose of various postnatal ages and 5-HT and 5-HIAA levels were measured by HPLC/EC one week after MDMA exposure. MDMA administration at or prior to postnatal day (PND) 10 did not result in deficits in 5-HT or 5-HIAA levels one week after MDMA exposure. Administration of 40 mg/kg of MDMA on PND 15 or PND 20 resulted in an 11 or 13 % depletion respectively, in 5-HT levels in the hippocampus, but had no significant effect on levels of 5-HIAA one week after MDMA exposure. This is in contrast to administration of a single dose of MDMA to 150 day old rats, wherein 5-HT and 5-HIAA Levels where decreased by 50 and 49%, respectively, in the hippocampus one week after MDMA exposure. These results indicate that serotoninergic neurons are sensitive to a long-term 5-HT depleting effects of MDMA as the rat CNS begins to mature. Further study of this phenomenon may provide information about the mechanisms responsible for the neurotoxic effects of MDMA.


Acquisition of the mature phenotype of neurons depends on both intrinsic and extrinsic signals. As part of a study of how these two signal sources interact during neural maturation in the medicinal leech, we have studied serotonergic neurons from segments containing the male and female reproductive ducts [Rc(R5)6] and from standard segments [Rc(R5)] applied to the soma. Single ganglia were isolated and desheathed, Rc neurons were voltage clamped with a single microelectrode and the ionic currents elicited by pressure pulses of ACh were studied. In addition, both types of Rc cells were studied in isolation in a cell culture system. All Rc cells responded to ACh with a phasic outward current, and Rc(X) in addition developed a rapidly desensitizing cationic inward current. This inward current had a physiological and pharmacological profile typical of neuronal nicotinic receptors. In contrast, the inward current possessed functional properties of which are typical of nicotinic cholinergic receptors and others of which are typical of muscarinic receptors. These distinct responses to the application of ACh mimic how each type of Rc cells responds when pressure-sensitive mechanoreceptor neurons (P cells) are stimulated. Therefore, we will determine whether the P-cell to Rc neuron synaptic interaction is mediated by a cholinergic synapse, as a model for the development of postsynaptic responses in segmentally homologous, but physiologically distinct, neurons that derive from the same cell lineage.

This work was supported by an NIH research grant (NS 25916 to WRK) and the International Human Frontier Science Program Organization (LS).

Tryptophan hydroxylase-immunoreactive neurons occur in the hypothalamus of the chick embryo. A.M. Gabaldon, J.K. Lobner, S.I. Sassere and J.A. Wallace*. Dept. of Physiology, Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131.

Numerous tyrosine hydroxylase-immunoreactive (TH+) cells occur in the chick hypothalamus in patterns observed for dopamine (DA)-containing neurons in mammals. However, the majority of these cells cannot be shown to contain DA. In chick embryos near hatching, we have now found the hypothalamus for the presence of cells immunoreactive for tryptophan hydroxylase (TPOH+), the first enzyme in the synthesis of serotonin (5-HT+). Large numbers of intensely stained TPOH+ neurons are found in locations contiguous with sites containing TH+ cells. However, TPOH+ cells occur in the paraventricular organ (PVO), no other site containing perikarya are observed in the hypothalamus corresponding to sites of TPOH+ neurons. This is in contrast to the lower brainstem where TPOH+ and 5-HT-immunoreactive cells co-localize in all nuclei examined. Therefore, most hypothalamic TPOH-neurons may lack amino acid decarboxylase or the isiform of the TPOH enzyme in these cells inactive. Supported by MBS grant GM-08139-18.

THE ROLE OF SEROTONERGIC REUPTAKE BLOCKADE IN PRODUCING COCAINE'S EFFECTS ON CEREBRAL FUNCTION IN PERIWEEING NTS. G.S. Frick, H.P. Hughes, B.A. Gross, L.A. Foret, D.I. Dow-Edwards Laboratory of Cerebral Metabolism, Department of Pharmacology, SUNY-Health Science Center at Brooklyn, Bklyn, NY 11203.

Previous studies from our laboratory have shown permanent neurochemical changes in rat brain followingcocaine postnatal days 11-20 (Dow-Edwards, Annals NY Acad Sci 562:280-289, 1989). By comparing the effects of fluoxetine, a serotonin reuptake inhibitor, to cocaine, the contribution of this pharmacologic property of cocaine to the long-term neurochemical effects can be determined. Sprague-Dawley rats were mated in our animal facility and on day of parturition, pups were assigned to receive sc injections of cocaine or fluoxetine at 25mg/kg or equivalent volume of each day during days 11-20. Local rates of glucose utilization were determined when the animals were 21 days old using the microPET scanner of the Ohio State PET Facility. The final dose of drug or vehicle was administered 20 min prior to deoxyglucose administration. ANOVA revealed a significant main effect of treatment in each of the 18 brain structures selected for analysis. Post hoc analysis indicated that cocaine but not fluoxetine administration resulted in altered patterns of brain glucose utilization. We therefore suggest that the effects of developmental cocaine exposure on adult brain function are not due primarily to inhibition of serotonin reuptake.

Supported by NIDA Grant DA04118 to DDE.
619.9 DEVELOPMENT OF NEUROTRANSMITTER METABOLISM IN THE BRAIN OF THE CHICK, GALLUS DOMESTICUS. Naokuni Takeka*, Dept. of Biotechnology, COSMO Research Institute, Saitte, Saitama, 340-01, JAPAN. The dynamics of neurotransmitters including precursor amino acids, biogenic monoamines and their metabolites were examined with progress of embryonic development. These 30 compounds were analysed simultaneously by coulometric three dimension HPLC method (CEAS: ESA Inc., USA). In the early stage (St. 5), putative pathways were TYR→TYR→HPA-4→TYR→L-DOPA→MD. In the middle stage (St. 10*), TRP→5HT→5HIAA→MD. In the late stage (St. 25*), NE was detected, in particular in the hindbrain. Just before hatching (St. 44*), DA→MD→DA→NE→DA increased with the progress of development. These main pathways are shown below. These results will be discussed from the viewpoint of ontogeny and phylogeny. (St. Hamburger & Hamilton, 1951) TRP→5HT→5HIAA→NE→DA→MD→NE→DA.

619.10 IMMUNOHISTOCHEMICAL EVIDENCE OF INDOLAMINE AND CATECHOLAMINE CONTAINING CELLS IN CULTURES OF HUMAN CENTRAL NERVOUS SYSTEM. M.C. Calvet*, C. Levallois. INSERM U336, USTL, 34095 Montpellier, France. Embryonic neurons from monoaminergic nuclei of the brainstem are used for neural transplantation; the knowledge of the localization and of the development of those embryonic cells has to be better defined. As previously described, serotonergic cells are mainly located in the rhombencephalon; catecholamine containing cells are located in the mesencephalon and an extensive system is described in the mesencephalon. The living dissociated cells isolated after 6-10 days in culture and immunostained for serotonin (5HT) and dopamine (DA) at different ages in vitro. Numerous 5HT-stained neurons in the rhombencephalon and DA-stained neurons in the mesencephalon were observed which developed numerous processes with well individualized growth cones. These results show that monoaminergic cells are characterized in the CNS at the early stages of human development.

ACKNOWLEDGEMENTS: Pr. J.L. Vialet, Prof. M. Geffard.

620.1 RESULTS FROM A RAPID SCREENING TECHNIQUE FOR ASSAY OF NEURONAL DIFFERENTIATION FACTORS. M.J. Farn*, P.H. Patterson. Division of Biology, 216-76, Caltech, Pasadena, CA 91125. We have developed a relatively rapid assay for simultaneously screening multiple neuronal differentiation activities. Sympathetic superior cervical ganglia are dissociated from neonatal rats and grown in 96-well plates in a chemically defined medium which contains various factors that may be important for neuronal development, such as IGF-I, EGF, SCF, oncostatin M (ONC), cholinergic neuronal neuropeptide or neurotransmitter synthetic enzyme mRNAs and candidate factors. After 7 days, total RNA is extracted and expression of mRNAs for 14 different neurotrophic factors and neurotransmitter synthesis enzymes is analyzed by parallel RTPCR method. Among the 21 cytokines and growth factors tested so far, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, INF-γ, TNF-α, GM-CSF, G-CSF, TGF-α, TGF-β, IGF-I, EGF, SCF, oncostatin M (ONC), cholinergic neuronal differentiation factor (CDF), ciliary neurotrophic factor (CNTF), PDGF, NGF, bFGF, only CDF and CNTF alter neuronal gene expression in this assay. This method in which both of these factors induce cholinergic and sympathetic proenkephalin mRNAs. Interestingly, although CDF, IL-6, and ONC are structurally related, and IL-6, CDF and ONC have receptor subunits in common, we are thus far unable to observe an induction of neuropeptide or neurotransmitter synthetic enzyme mRNAs by IL-6 or ONC in the sympathetic neurons.

620.2 QUANTIFICATION OF mRNA LEVELS ENCODING NEUROTROPHIC FACTORS DURING POSTNATAL DEVELOPMENT OF MOUSE NIGROSTRATIAL DOPAMINE NEURONS. Cynthia L. St. John and Maria Dun. Fishberg Research Center for Neurology, Mont Sinai School of Medicine, New York, NY 10029. Neuritrophic factors in the EGF, FGF and neurotrophin family have been shown to increase the survival of midbrain dopamine neurons in vitro. Conventionally, trophic molecules are thought to be provided by targets to support projection neurons. Not only is there evidence for the expression of growth factors in the substantia nigra and even in the dopamine neurons themselves. These trophic substances may be acting as target-derived factors and serve as paracrine or autocrine factors to promote growth and survival. In order to substantiate the role of neurotrophic factors in the development and maintenance of dopaminergic neurons, we have begun a postnatal developmental study on the expression of EGF, TGF-α, BDNF, IGF-1, IGF-2, and insulin. mRNA in the dorsal striatum and ventral mesencephalon. Brain tissue was collected at fourteen postnatal time points ranging from two days to twenty weeks. The quantitative nucleic acid extraction assay was employed to determine the levels of growth factor mRNA in total cortical mRNA. The specific mRNA for EGF, IGF-1, IGF-2, and IGF-1 were detected at every time point assayed and were compared after normalization to total RNA. Postnatal developmental TGF-α mRNA levels were consistently higher in the dorsal striatum than in the ventral mesencephalon; whereas BDNF mRNA levels were always higher in the ventral mesencephalon. The expression level of IGF-1 increases in both areas as the animals matures. The advantage to this approach is that within a defined region, at a given time in development, growth factor levels can be compared and relationships between growth factor family members can be identified. Correlating the changes in expression of these neurotrophic factors and known developmental events will give clues to the function of these important molecules in normal brain development.

620.3 MODEL NEURONAL CULTURES TO STUDY THE EFFECTS OF DRUGS ON NEURONAL FUNCTION. W. Chen, P. Coates and E. Podolsky. Dept. of Neurology, Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX 79430. Monolayer cultures of neurons were prepared from midbrain and striatal areas of 5-day-old rat embryos using a trypsin-EDTA procedure. The specific brain areas were dissected into 2 mm3 tissue blocks, subjected to a brief trypsinization step in isolation medium. The softened tissue was passed through nylon screens and the cell suspension plated onto poly-hydroxyethyl methacrylate coated flasks. After one hour, the media was changed to glucose with insulin, 1% BSA and 10% fetal calf serum) was changed to a maintenance of dopamine neurons, we have begun a postnatal developmental study on the expression of EGF, TGF-α, BDNF, IGF-1, IGF-2, and insulin. mRNA in the dorsal striatum and ventral mesencephalon. Brain tissue was collected at fourteen postnatal time points ranging from two days to twenty weeks. The quantitative nucleic acid extraction assay was employed to determine the levels of growth factor mRNA in total cortical mRNA. The specific mRNA for EGF, IGF-1, IGF-2, and IGF-1 were detected at every time point assayed and were compared after normalization to total RNA. Postnatal developmental TGF-α mRNA levels were consistently higher in the dorsal striatum than in the ventral mesencephalon; whereas BDNF mRNA levels were always higher in the ventral mesencephalon. The expression level of IGF-1 increases in both areas as the animals matures. The advantage to this approach is that within a defined region, at a given time in development, growth factor levels can be compared and relationships between growth factor family members can be identified. Correlating the changes in expression of these neurotrophic factors and known developmental events will give clues to the function of these important molecules in normal brain development.

620.4 ANALYSIS OF SERUM ANTI-GM1 ANTIBODY TITER IN PATIENTS RECEIVING GM1 THERAPY. R.K. Yu*, M. Salto, Y. Zhang, R. Fiorentini, Y. Khin-Maung-Gyi. Friday Med. Coll. VA, VCU, Richmond, VA 23298 and Fidia Pharm. Corp., Washington DC 20006. It is well known that GM1 gangliosides, particularly GM1, possess neurotrophic and neuroprotective properties in vitro and in vivo. Gangliosides have also been shown to exert protective effects in certain neurodegenerative conditions, including strokes, spinal cord injury, and neuropathies. Thus, ganglioside GM1 is used as a therapeutic agent for a variety of neurological diseases. To examine the safety of GM1 therapy, we conducted double blind placebo controlled studies in normal subjects and patients with acute ischemic cerebral infarction. Serum samples removed at different time interval (day 0 to 84) were assayed for anti-GM1 titers using a solid-phase ELISA. Of the 418 samples assayed, only 7 were judged to have a marginal anti-GM1 response at 1:800 dilution. Among the 7 positive samples, 1 was from a subject who received a single dose (1,200 mg) of GM1 for a subject before and after receiving 400 mg of GM1, and the other 4 were from 3 subjects receiving no therapy. We conclude that there is no clear association between GM1 therapy and the development of anti-GM1 titers.

OTHER FACTORS AND TROPHIC AGENTS: GENERAL II

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
**620.5**

**COMBINED BUT NOT INDIVIDUAL GROWTH SUBSTANCES MIMIC THE REPARATIVE EFFECT OF GM1 GANGLIOSIDE ON CULTURED DOPAMINE NEURONS DAMAGED BY MPP⁺.** N. Stull* and L. Iacovitti. Dept. of Neurology, Thomas Jefferson University, Philadelphia, PA 19107

We have previously demonstrated that, in culture, GM1 ganglioside can increase the number of dopamine neurons surviving MPP⁺ toxin treatment as well as enhance their biochemistry (Stull et al., 1991). Cytokine and neuronal growth factor factors are also thought to play a role in the survival and biochemical development of these neurons. Therefore, we sought to determine the effects of individual growth substances either individually or in combination to better enhance the reparative effects of GM1 on damaged dopamine neurons. The ventral mesencephalon was dissected, plated, and maintained on standard media. After 3 days, some cultures were fed media containing 2.5 μM MPP⁺. One day later, cultures were refed media supplemented with one or all of the following: NGF, BFGF, kFGF, IGF, CNTF, NT3, IGF, EGF, FGF, and insulin (1-100μg/ml). To test whether growth substances could further enhance the GM1 effect, some media was simultaneously supplemented with 100μM GM1. After 7 days in vitro, cultures were processed for assay of tyrosine hydroxylase (TH) activity and TH-immunostaining. Exposure to MPP⁺ for 24 hours produced a 23-30% decline in the number of surviving TH+ neurons. The addition of individual growth substances had no effect on cell loss due to MPP⁺. However, the addition of a mixture of all growth substances to MPP⁺-treated cultures restored the number of TH+ neurons to nearly control levels (99%), and simultaneously induced the level of TH activity/TH+ neuron (33%). Induction by TFGβ alone resulted in a comparable degree of biochemical induction (35%) but had no effect on dopamine cell survival. Addition of GM1 to individual growth substances or the mixture did not further enhance survival or enhance dopamine biochemistry. These data suggest that while individual factors like TFGβ may influence transmitter biochemistry, it is the combination of growth substances which may be important for improving dopamine cell survival after MPP⁺ damage. Since GM1 does not act synergistically with these substances, it is thought that GM1 and both substances through a common pathway to achieve their beneficial response in injured dopamine neurons.

**620.7**


We have designed a 30 mer oligonucleotide representing a common and conserved heparin-binding sequence of NGF and BDNF to screen a cDNA library constructed in plasmid pGEM, from poly(A)-RNA extracted from lesioned rat brain. More than 30 clones with a positive signal were sequenced with T7DNA polymerase from both ends. One of the above clones appears to be potentially interesting in light of the trophic and/or adhensive activity of the gene product(s). Sequence comparison with a peptide from the extracellular segment (1000bp-1250bp) revealed homology from both ends, of 53% and 55% with β2 laminin chain, and of 67% with IGF over 130 bp in the middle of the insert. In addition, G41 has a motif representing 100% homology to N-CAM. We have previously shown that in the rat, the unique hybridizing band was detected at a position corresponding to approximately 3.8kb and that G41 gene is regulated during brain injury. A unique band with an apparent similar size was observed in mouse brain. In the present study, we examined the developmental pattern of G41 expression in normal and mutant mouse brain. We identified, by western blot, a protein of 60kDa. The expression of the protein was found to be tissue specific, having a higher expression in the brain. The protein was found to be more abundant in the cortex and basal ganglia than in the cerebellum. The protein was also found to be expressed in the hypothalamus and olfactory bulb. The expression of the protein was found to be downregulated in the cortex of a mouse mutant with a defect in the development of the basal ganglia. The expression of the protein was also found to be downregulated in the cortex of a mouse mutant with a defect in the development of the basal ganglia. The expression of the protein was also found to be downregulated in the cortex of a mouse mutant with a defect in the development of the basal ganglia. These results suggest that G41 is a novel laminin-like protein that may play a role in the development of the central nervous system.

**620.8**

**EFFECT OF CONCANAVALIN A (Con A) ON GROWTH OF CULTURED NEURONS FROM MAMMALIAN CENTRAL NERVOUS SYSTEM (CNS).** P.W. Coates* and T.L. McKee. Cell Biology & Anatomy, Texas Tech Univ. HSC- School of Medicine, Lubbock, TX 79430.

The lectin Con A is a useful tool for inducing alterations in properties of some cultured neurons. Besides changing membrane properties (ion channel activity, neurotransmitter responses and synaptic connectivity), Con A promotes nerve fiber outgrowth in Aplysia, leech and chick dorsal root ganglia. To determine whether neurons from mammalian CNS grow more in response to Con A, neurons from fetal rat brain (striatum) were cultured at low cell density in three-dimensional (3D) collagen lattices or polyethyleneimine (PEI) coated dishes in the presence or absence of Con A (0.5 or 50 μg/ml). Fiber length of single neurons was measured using computerized image analysis. Morphological indicators of complex growth were also assessed. Data from three experiments were pooled and analyzed using analysis of variance. Total nerve fiber length of single neurons increased significantly for neurons cultured in low dose Con A on 3D. Fiber outgrowth of neurons on PEI showed a similar pattern. At higher Con A concentration, there was an increase in mean number of primary processes for neurons on both substrates, and in average number of terminals of neurons on PEI. Observations suggest that neurons from mammalian CNS can grow longer nerve fibers and express somewhat more complex growth patterns under the influence of Con A. The precise molecular mechanism by which Con A induces alterations in neuronal growth (or membrane properties) is not known, but it has been suggested that different mechanisms might be involved rather than a single receptor and transduction mechanism. It is also conceivable that under these culture conditions Con A could affect mechanisms for fiber elongation differently than mechanisms influencing complex growth. Supported by a grant from the National Institutes of Health.

**620.9**

**TROPHIC ACTIONS OF MEDIUM (MK), A HEPARIN-BINDING PROTEIN, ON MOUSE SPINAL CORD AND SENSORY GANGLION AFFERENTS IN CULTURE.** N. Michihaus, M. Nishimura, N. Muramatsu and S.U. King. Dept. of Neurology, Univ. of British Columbia, Vancouver, Canada; and Dept. of Biochemistry, Kagoshima Univer., Kagoshima, Japan

Medium (MK) is a 14 kDa protein product of a retinoic acid responsive gene, MK, and is a member of a new family of heparin binding growth factors structurally unrelated to FGF. J. Cell Biol 110, 407, 1990). It has a 50% sequence homology with pletrothrophin, a growth factor involved in cell migration in 13-14 day fetal cerebral cortex treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment has been found to promote neurite extension in embryonic rat CNS neurons. Trophic effects of MK were studied in dissociated cell cultures of embryonic rat spinal cord and dorsal root ganglion (DRG). There was a 3-4 fold increase in the number of MAP-2 immunoreactive neurons and a 2-4 fold increase in choline acetyltransferase activity (ChAT) in MK-treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment has been found to promote neurite extension in embryonic rat CNS neurons. Trophic effects of MK were studied in dissociated cell cultures of embryonic rat spinal cord and dorsal root ganglion (DRG). There was a 3-4 fold increase in the number of MAP-2 immunoreactive neurons and a 2-4 fold increase in choline acetyltransferase activity (ChAT) in MK-treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment has been found to promote neurite extension in embryonic rat CNS neurons. Trophic effects of MK were studied in dissociated cell cultures of embryonic rat spinal cord and dorsal root ganglion (DRG). There was a 3-4 fold increase in the number of MAP-2 immunoreactive neurons and a 2-4 fold increase in choline acetyltransferase activity (ChAT) in MK-treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment has been found to promote neurite extension in embryonic rat CNS neurons. Trophic effects of MK were studied in dissociated cell cultures of embryonic rat spinal cord and dorsal root ganglion (DRG). There was a 3-4 fold increase in the number of MAP-2 immunoreactive neurons and a 2-4 fold increase in choline acetyltransferase activity (ChAT) in MK-treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment has been found to promote neurite extension in embryonic rat CNS neurons. Trophic effects of MK were studied in dissociated cell cultures of embryonic rat spinal cord and dorsal root ganglion (DRG). There was a 3-4 fold increase in the number of MAP-2 immunoreactive neurons and a 2-4 fold increase in choline acetyltransferase activity (ChAT) in MK-treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment has been found to promote neurite extension in embryonic rat CNS neurons. Trophic effects of MK were studied in dissociated cell cultures of embryonic rat spinal cord and dorsal root ganglion (DRG). There was a 3-4 fold increase in the number of MAP-2 immunoreactive neurons and a 2-4 fold increase in choline acetyltransferase activity (ChAT) in MK-treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment has been found to promote neurite extension in embryonic rat CNS neurons. Trophic effects of MK were studied in dissociated cell cultures of embryonic rat spinal cord and dorsal root ganglion (DRG). There was a 3-4 fold increase in the number of MAP-2 immunoreactive neurons and a 2-4 fold increase in choline acetyltransferase activity (ChAT) in MK-treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment has been found to promote neurite extension in embryonic rat CNS neurons. Trophic effects of MK were studied in dissociated cell cultures of embryonic rat spinal cord and dorsal root ganglion (DRG). There was a 3-4 fold increase in the number of MAP-2 immunoreactive neurons and a 2-4 fold increase in choline acetyltransferase activity (ChAT) in MK-treated spinal cord (13-14 day fetal) cultures. The number of surviving neurons following MK treatment has been found to promote neurite extension in embryonic rat CNS neurons.

Using a novel smooth muscle (SM) preparation, the avian amin, we have examined the extracellular factors that determine the responsiveness of SM cells to specific neurotransmitters. The avian amin is an anatomically simple tissue, neither innervated nor vascularized, containing only a single layer of epithelium, and 1-2 layers of SM. While responsive to many types of neurotransmitter, the avian amin is normally unresponsive to VIP. This is unusual because most non-vascularized SMs contract vigorously in response to nonanomalous SM cells. The avian amin sprouts in a manner similar to that of nonanomalous SM cells, but does not in direct contact with the purified basement membrane, showed the usual induction of substance P responsiveness. The data suggest that extracellular basement membranes normally suppress the avian's response to substance P. We hypothesize that other tissues expressing diverse pharmacological phenotypes may also be regulated by molecules in the extracellular matrix.

620.13 REGULATION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) AND SUBSTANCE P (SP) EXPRESSION IN SUPERIOR CERVICAL GANGLION (SCG) AFTER AXOTOMY AND IN ORGAN CULTURE. M. S. Rao, T. Y. Ho, J. Valiyaveettil, S. L. Gluck, and R. E. Zigmond. Dept. of Neuroscience, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

The expression of VIP and SP, previously shown to be affected by axotomy, was assessed in SCG after axotomy and in dissociated cells maintained in culture. In addition, VIP-IR and SP-IR increases in SCG in situ within 45 h of postganglionic axotomy (Brain Res. 1740:1991). We have examined the time course of VIP induction after axotomy and found that levels of VIP-IR increased significantly by 24 hr, reached a peak by 6 days, and remained elevated for at least 2 weeks. To determine whether VIP is also increased by axotomy, we measured SP-IR 48 hr and 2 weeks after surgery, using a radioimmunoassay. Consistent with previous report (Brain Res. 234:182,1982), there was no difference in the level of SP-IR between axotomized and sham-operated ganglia 2 weeks after injury; however, 48 hr after axotomy, SP-IR was increased 12-fold. Immunohistochemical studies revealed an increase in SP-IR in principal neurons in the SCG 48 hr after axotomy. Previous studies have indicated that the increase in substance P occurs in organ-cultured SCG can be reduced by the synthetic glucocorticoid dexamethasone. We have compared the effects of dexamethasone (0.1 μM) on VIP and SP expression in organ culture and in cell culture. Dexamethasone blocks the increases in SP-IR normally seen in both types of culture almost completely and reduced the increases in VIP-IR by about half. Thus, qualitatively there are marked similarities in the regulation of VIP and SP in the SCG, though there appear to be quantitative differences. (Supported by NS12651, MH01662, HD25561 and a AHA fellowship to MSR.)

620.15 REGULATION OF VASOACTIVE INTESTINAL PEPTIDE (VIP) EXPRESSION IN RAT SUPERIOR CERVICAL GANGLION (SCG) IN ORGAN CULTURE BY AGENTS THAT ELEVATE CAMP. R. P. Mohney and R. E. Zigmond*. Dept. of Neuroscience, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

VIP expression increases in sympathetic neurons in adult rat SCG in organ culture. The VIP gene contains a CAMP response element that is required for CAMP-regulated transcription of the gene. VIP itself and secretin, another member of the same peptide family, have been shown to act in many tissues as a potent cAMP inducer. Therefore, we examined whether these peptides can increase VIP expression in the SCG. Adult rat SCG explants were cultured for 30 min in medium containing 500 μM IBMX or 150 μM forskolin or 5 μM secretin. Both forskolin and secretin significantly increased CAMP levels in the ganglia. After 24 or 48 hr in culture, explants treated with forskolin or secretin also showed a significant (5-fold) increase in VIP-like immunoreactivity (IR). In contrast, 10 μM isoproterenol increased CAMP levels without affecting VIP-IR. This apparent discrepancy may be due to isoproterenol causing changes in CAMP levels in non-neuronal cells, but not in neurons, in the ganglion. Peptide histidine isoleucine amide (PHI) is coded for by the same mRNA that codes for VIP. Preliminary data indicate that 10 μM VIP increases the level of PHI-IR. The data raise the possibility of a positive feedback loop in which VIP could stimulate its own synthesis.


Previous studies have indicated that VIP is a multifunctional regulator of developmental and survival during a critical embryonic period in the rat (Pincus et al. Nature 343:564). Moreover, the local production of VIP in sympathetic ganglia in vivo suggested that the peptide acts via autocrine mechanisms (Pincus et al., Soc. Neuosci. 12:806, 1986). To define the spectrum of populations responsive to VIP mitogenic and trophic activity, we examined effects in developing sensory and cerebellar granule neurons. Dissociated embryonic day 14.5 dorsal root ganglion cells were preplated to reduce non-neurons and cultured in serum-free media with NGF for 48 h. The major neuronal populations were enriched for sensory or cochlear small round or oval cells exhibiting mono- or bipolar neurites. VIP elicited a two-fold increase in neuron number. Neurons cultured in the presence of VIP, however, did not incorporate [3H]thymidine, a marker for DNA synthesis, suggesting that the peptide was not mitogenic. To the contrary, cell counting indicated that VIP elicited increased neuronal survival. VIP trophic activity was specific, since several related peptides were without effect. While NGF was permissive for VIP effects, peptide trophic activity did not depend on the presence of the neurotrophic or non-neurons. In contrast to sensory neurons, VIP exhibited only mitogenic activity in cultures of postnatal day 7 cerebellar granule cells. The data suggest that VIP effects in cultures have their action in different neuronal populations. (Dysautonomia Fdn, NIH-HD23315, UMDNJ Fdn)
620.17

Pituitary Adenylate Cyclase-Activating Peptide (PACAP) is a new member of the vasoactive intestinal peptide/glucagon/secretin peptide family. PACAP binds with high affinity to cells of the peochromocytoma cell line PC12h and stimulates adenylate cyclase (Watanabe et al., BBRC 172-252, 1990). In the present study we examined some of the neurotrophic properties of PACAP on PC12 cells including their metabolism, cell survival and neurite outgrowth. PC12 cells were maintained in RPMI medium with 10% inactivated serum, 150 ng/ml nerve growth factor (NGF), and 5% fetal bovine serum. At 10 nM PACAP caused a 5-fold and 1.3-fold increase in cell diameter and cell protein respectively. Cell survival was determined in serum free medium in the absence and presence of PACAP from 0.01 to 100 nM and NGF at 2 nM. After 5 days the following levels of survival were determined relative to the initial number of cells plated: serum free control, &lt;20%, NGF, 88%, PACAP 0.3 to 3 nM, 60% and 10 to 300 nM 100%. In the presence of 1% horse serum, 10 nM PACAP27 supported neurite outgrowth from 70% of the cells at a nearly linear rate of 7.5 mm/day. Neurites possessed varicosities and terminal growth cone-like structures and were stable for up to two weeks. Thus, the hypothesis that PACAP is a new neurotrophic factor is supported by the localization of PACAP and its receptor to both the central and peripheral nervous systems and the data presented here demonstrating PACAP's capacity to regulate the metabolism, survival, and morphological differentiation of PC12 cells.

620.18

Pituitary Adenylate Cyclase-Activating Peptide (PACAP) is a new member of the vasoactive intestinal peptide/glucagon/secretin peptide family of peptides which binds with high affinity to peochromocytoma cells (PC12h) and stimulates adenylate cyclase (Watanabe et al., BBRC 172-252, 1990). PACAP27 was recently shown to act as a neurotrophic factor by increasing PC12 cell diameter and total protein, enhanced cell survival in low serum medium and promoted neurite outgrowth (see Weil, et al., 1992 Soc for Neurosci Abstr.). We have examined the induction of several immediate early genes in cultured PC12 cells treated with PACAP27 and with nerve growth factor (NGF) by employing a modified nuclear run-on procedure involving the rapid extraction and column purification of mRNA. PACAP induced a different pattern of gene expression than did NGF: whereas 2nM NGF enhanced the level of expression of several genes (c-fos, b-actin, jun-jun-myc), 10nM PACAP most noticeably enhanced c-fos, but had little effect on c-myc, c-jun or b-actin expression at 30 min. A dose response study showed maximal c-fos and c-jun induction at 10 min, while b-actin was slightly induced later at 1-2h. Thus, PACAP was shown to regulate the transcription of immediate early genes in PC12 cells which further supports the hypothesis that PACAP is a new neurotrophic factor.

620.19
INNERVATION DEPENDENT PRODUCTION OF CHOLINERGIC DIFFERENTIATION FACTOR BY SWEAT GLAND CELLS. S.J. Tresser, M.S. Beo, and S.C. Lands. Deps. of Neurological Surgery and Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

During normal development the sympathetic innervation of rat sweat glands undergoes a switch from a noradrenergic to cholinergic phenotype. Transplantation experiments indicate that the switch is induced by the target tissue. Previous studies have shown that cholinergic inducing activity that could be responsible for directing the switch is present in extracts of developing and adult rat footpads. In the present studies we have examined whether this activity is due to sweat gland cells and begun to study its regulation. First, homogenates of footpads of tabby mutant mice which lack sweat glands were tested for their ability to induce choline acetyltransferase (ChAT) activity in cultured sympathetic neurons. Levels were significantly reduced, suggesting that most of the activity in normal footpads is associated with the glands. Second, primary cultures of rat sweat gland cells were grown in the absence of neurons. Co-culture with neurons, however, resulted in a twelve-fold increase in the amount of sympathetic ChAT activity in cultured sympathetic neurons. Levels were significantly reduced, suggesting that most of the activity in normal footpads is associated with the glands. This switch was shown to be due to sweat gland cells by comparing lesioned to sham-operated animals. We have observed an increase in taurine-like immunoreactivity (Tau-LI) in the outer molecular layer of the cerebellum of 7 day old rats, which could indicate the function(s) of taurine. These changes were present at 3, 7, 14, 28 and 56 days post lesion. The increase in Tau-LI within the molecular layer appeared to be associated with the region of increased ACHE staining. Increases in Tau-LI were also seen in the ipsilateral stratum lenticularis of CA1, which is another terminal zone for the perforant pathway. Increases in glutamate-like immunoreactivity (Glu-LI) were observed in the outer molecular layer of the dentate gyrus ipsilateral to the lesion in some animals. No changes were observed in Glu-LI in the stratum lacunosum/moleculare. These results suggest that taurine may play a role(s) in the process of regeneration in the young adult brain, but these roles are not always linked with changes in glutamate.

621.1
CHANGES IN TAURINE-LIKE IMMUNOREACTIVITY DURING DEVELOPMENT OF THE HIPPOCAMPUS AND CEREBELLUM OF THE RAT. Kathy R. Magnusson* & Dept. of Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523

Taurine, which is known to be involved in development of the nervous system, is protective against glutamate toxicity. Our goal was to determine whether these neuroactive amino acids are linked during regeneration by examining changes in immunohistochemical staining in the dentate gyrus following unilateral electrolytic lesion of the entorhinal cortex. With the use of monoclonal antibodies, changes in immunoreactivity were determined by comparing lesioned to sham-operated animals. We have observed an increase in taurine-like immunoreactivity (Tau-LI) in the outer molecular layer of the dentate gyrus, ipsilateral to the lesioned entorhinal cortex in 2 month-old rats. These changes were present at 3, 7, 14, 28 and 56 days post lesion. The increase in Tau-LI within the molecular layer appeared to be associated with the region of increased ACHE staining. Increases in Tau-LI were also seen in the ipsilateral stratum lenticularis of CA1 which is another terminal zone for the perforant pathway. Increases in glutamate-like immunoreactivity (Glu-LI) were observed in the outer molecular layer of the dentate gyrus ipsilateral to the lesion in some animals. No changes were observed in Glu-LI in the stratum lacunosum/moleculare. These results suggest that taurine may play a role(s) in the process of regeneration in the young adult brain, but these roles are not always linked with changes in glutamate.

621.2
CHANGES IN TAURINE- AND GLUTAMATE-LIKE IMMUNOREACTIVITY DURING REGENERATION IN THE DENATE GYRUS OF THE RAT. Catherine Tannert, Tim D. Hassinger* and Kathy R. Magnusson. Dept. of Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523

The results suggested that dopamine serves as a stop signal for axons, prior to synapse formation. Taurine, which is known to be involved in development of the nervous system, is protective against glutamate toxicity. Our goal was to determine whether these neuroactive amino acids are linked during regeneration by examining changes in immunohistochemical staining in the dentate gyrus following unilateral electrolytic lesion of the entorhinal cortex. With the use of monoclonal antibodies, changes in immunoreactivity were determined by comparing lesioned to sham-operated animals. We have observed an increase in taurine-like immunoreactivity (Tau-LI) in the outer molecular layer of the dentate gyrus, ipsilateral to the lesioned entorhinal cortex in 2 month-old rats. These changes were present at 3, 7, 14, 28 and 56 days post lesion. The increase in Tau-LI within the molecular layer appeared to be associated with the region of increased ACHE staining. Increases in Tau-LI were also seen in the ipsilateral stratum lenticularis of CA1, which is another terminal zone for the perforant pathway. Increases in glutamate-like immunoreactivity (Glu-LI) were observed in the outer molecular layer of the dentate gyrus ipsilateral to the lesion in some animals. No changes were observed in Glu-LI in the stratum lacunosum/moleculare. These results suggest that taurine may play a role(s) in the process of regeneration in the young adult brain, but these roles are not always linked with changes in glutamate.

Post-crush retinal explants have been used to study the regulation of the outgrowth by neuroactive agents. Taurine has a trophic effect on this retina in culture and in vivo, possibly mediated by the entrance of calcium (Lima et al., 1986, 1991). The serotoninergic receptors, the tranylcypromine and the modulation of the serotoninergic system by light stimulus has been also studied in this retina (Lima et al., 1992; Schmeer et al., 1991). Serotonin (5HT) has a selective effect on the development of neurons of invertebrates and vertebrates (Murray et al., 1990; Strich et al., 1990). The nerve growth index of retinal explants was evaluated as the product of fiber length and density. Monamines and metabolites were determined by HPLC with EC. Serotonin (5HT) at nM concentrations completely blocked the regeneration and antagonized the stimulatory effect of taurine. B-Dih-Dipropylaminotetraethylpen (DPTP), a 5HT receptor agonist had a similar effect. The 5HT receptor agonist, (+)-1,2,5dimethoxy-4-iodophenil-2-iminopropanol (IMIP) and serotonin uptake blockers, such as imipramine and fluoxetine were less potent in the impairment of outgrowth. The concentration of 5HT decreased 3 days and 5 days after the crush of the optic nerve, and taurine play a role in the regeneration of the post-crush retina.

Grant SI-2228 - CONICIT

621.5

CHRONIC NEONATAL NMDA RECEPTOR BLOCKADE WITH MK-801 ALTERS MONOAMINE METABOLISM AND AFFECTS SPATIAL LEARNING IN THE ADULT RAT. J.A. Gomez*, G.J. Boer, M.G.P. Fonsestra, M.H.A. Botterblom and J.P.C. de Bruin. Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands (Spon: ENA)

We examined the question whether chronic MK-801 treatment in neonatal rats (0.25 mg/kg i.p. per day from postnatal day 8 through 19), which previously had been shown to alter NMDA receptor function, would also affect spatial learning in the adult rat. MK-801 treated rats were able to learn the spatial (water maze) task with no control rats at a significantly slower rate. Visual cue learning was not affected by the neonatal treatment, indicating that the slower spatial learning is not caused by either locomotor or sensory deficits. Although MK-801 injections induced inhibitory motor activity throughout the treatment period, in adulthood there were no longer differences apparent. At the same time, however, monoamine metabolism of the frontal cortex and striatum as detected by high pressure liquid chromatography with electrochemical detection, was higher in the MK-801 treated rats, while markers for GABAergic neurons, astrocytes and myelination were at the same levels as controls. No significant neurochemical differences were noticed in the cortex and hippocampus except for delayed maturation of the myelination marker. The treated rats, tested with astrometric cages, showed a marked hyperactivity which lasted for at least 2 weeks after the end of the treatment. Data on adult rats treated in the same way, will be presented.

621.6

STIMULATION OF SOMATOSTATIN EXPRESSION IN CULTURED CILIARY GANGLION NEURONS BY ACTIVIN IN CHOROID CELL CONDITIONED MEDIUM. J.N. Coulombe1, E. Schwamb3, A.S. Parent3, F.P. Eckenstein3, and R. Nishi3. 1Dept. of Anatomy, Uniformed Services University of the Health Sciences, Bethesda MD 20814; 2Dept. of Endocrinology, Genentech Inc., So. San Francisco CA 94080; 3Dept. of Cell Biology & Anatomy, Oregon Health Sciences University, Portland OR 97201.

We are interested in testing the hypothesis that neurotransmitter phenotype can be regulated by interactions between neurons and the targets they innervate. The chicken ciliary ganglion (CG) contains two populations of neurons: ciliary neurons that innervate striated muscle in the iris and ciliary body and choroid neurons that innervate vascular smooth muscle in the choroid layer of the eye. Ciliary and choroid neurons differ in their transmitter phenotype in that choroid neurons use the neuropeptide somatostatin as a co-modulator with acetylcholine. We have previously shown that CG neurons are induced to express somatostatin-like immunoreactivity (SOM-IR) by co-culture with choroid cells. This interaction is mediated by a macromolecule in choroid conditioned medium (Coulombe & Nishi, J. Neurosci. 15: 533). Here we present evidence that this somatostatin stimulating activity is likely to be activin A, a molecule that has been implicated in a variety of other developmentally important phenomena. Our results are the following: 1) human recombinant activin A induces SOM-IR in cultured CG neurons; 2) ChCM mimics activin induction by inducing hemoglobin synthesis in K562 cells; 3) hemoglobin synthesis induced in K562 cells by ChCM is inhibited by inhibin; 4) western blot analysis of ChCM with activin A specific antibodies reveals one band that co-migrates with human activin A; 5) northern blot analysis of poly-A+ RNA from cultured choroid cells reveals a band that hybridizes at high stringency with an activin A (inhibin) subunit riboprobe; 6) follistatin, an activin-binding and inactivating molecule, abolishes the ability of ChCM to induce hemoglobin in K562 as well as its ability to induce SOM-IR in CG neurons. These results suggest that activin may serve as a target-derived factor controlling somatostatin in vivo. Supported by NS25767 (RN), AG07424 (FPE), EY06178 (JNC), and EY06352 (ASP).

621.7

SOMATOSTATIN ENHANCES NEURITE OUTGROWTH IN PC12 CELLS. Dolores Ferrero*, R. Ann Sheldon, Robert Messing.

Somatostatin (SS) is present in selective regions of the developing central nervous system in very high quantities suggesting a role for the peptide in neuronal differentiation. SS is in high concentrations in the human retina and can stimulate neurite outgrowth in molluscan neurons. We have studied the effects of SS on neurite outgrowth in a well defined model of neuronal differentiation, PC12 cells after Nerve Growth Factor (NGF) induction. Using this model we have found that SS can enhance the NGF induced neurite outgrowth. We plated PC12 cells at a density of 10 4-10 5 cells/well and examined cells every 24 hours for neurite formation after incubation with NGF. A neurite was defined as a process greater than one cell body in diameter in length possessing a terminal growth cone. The percentage cells bearing neurites were counted by counting 100 cells/well in quadruplicate wells. Cells plated on polylysine coated dishes grown in defined medium containing 50mCi NGF began to develop distinct neurites on day 4. Similarly, we found an increase in the percentage of neurite-bearing cells significantly (NGF 70±% vs. SS 84±%: p<0.03). On day 2 using 10 μg/ml concentrations of NGF (1-4M), a marked increase in the number of cells bearing neurites was seen in the presence of 1μM SS (70±% vs. 97±%: p<0.01). On inspection of the cells, those treated with SS + NGF had significantly longer neurites than the untreated cells (3.8±μm ±1.0 vs. 1.8±μm: p<0.01). In addition, the complexity of neurite networks was greater in the SS + NGF treated cultures. These preliminary data suggest that SS may function as a trophic factor in early neuronal differentiation, promoting neurite outgrowth.

621.8

THYROID HORMONE ALTERS CHOLINERGIC SOMAL ENLARGEMENT AND RECOVERY OF CHAT+ NEURON NUMBER AFTER AXONOTOMY OF PROJECTIONS FROM BASAL FOREBRAIN TO MEDIAL CORTEX. T.W. Farris* and L.L. Butcher. Laboratory of Chemical Neuroanatomy and Dept. of Psychology, UCLA, Los Angeles, CA 90095.

To examine the regenerative morphologic effects of putative growth-promoting factors on cholinergic neurons, we administered thyroxine (T4, 2.5 mg/kg, ip) or saline to rats for 15 days at 21 days of age. This treatment resulted in 80±% reduction of female rats following unilateral knife-cut axotomies of the cholinergic medial pathway which projects from the cholinergic basal nuclear complex (CBNC) to the cingulate and occipital medial cortices. Brain tissue was processed immunohistochemically for choline acetyltransferase (ChAT) and for Nissl substance. In the saline groups, computerized morphometry using light microscopy showed surprising recovery of medial septum (MS) somata—which did not project appreciably to medial cortex and, thus, were not axotomized—underwent mild transient enlargement at 30 days compared to 7 and 60 days (p < 0.05). Yet, no effect was seen in soma of the vertical limb of the diagonal band (VDB) which do project to cortex and were axotomized. Concomitantly, maximal loss of CHAT+ somal number on postlesion days 7 (for MS) and 15 (VDB) recovered by day 60 (p < 0.05 each). No change in somal number was seen in Nissl-stained sections possibly indicating only altered CHAT expression. Thyroxine treatment from day 30 to 15, and peak VDB CHAT+ neuronal number from day 60 to 15. These data imply that adult CBNC neurons, fated metabolically by a sublethal axotomy, appear to undergo recovery in response to thyroid hormones. [Support: NIH NS 10928 to L.L.B.]
621.9

Epidermal growth factor (EGF) induces the neuronal differentiation of PC12 cells in that it causes the extension of short neurites. The protein kinase inhibitor K252a blocks NGF-induced neurite outgrowth, but it potentiates the EGF effect, resulting in long branched neurites comparable to those induced by NGF. The related compound, K252b which cannot permeate cell membranes, inhibits NGF-induced differentiation, but it neither inhibits nor potentiates EGF-induced neurite outgrowth suggesting that K252a produces its potentiating effect on EGF by acting at an intracellular site. RNA synthesis and activity of the raf proto-oncogene product are required for neurite outgrowth induced by EGF or by a combination of EGF and K252a. EGF increases the mRNA levels of two late response genes (SCG10 and 63) that had previously been found to be induced in PC12 cells only by NGF and K252a. K252a increases the EGF-induced expression of these two genes. These results suggest that EGF and NGF trigger similar but not identical signal transduction pathways.

621.10
EGF INCREASES THE NUMBER OF PROLACTIN CELLS IN NEONATAL RAT PITUITARY CULTURES. E. Felix, A. Navarrete, A. Marin, and G. Cota*. Department of Neurosciences, Clínica Las Condes, Santiago, Chile, 760000

It is known that chronic treatment of rat pituitary tumor cells with EGF stimulates prolactin production. We investigate the effect of the action of EGF on the prolactin cell population of neonatal (10-day-old) male rats. Anterior pituitary cells were cultured for 2 days, in the absence or presence of 5 nM EGF in the culture medium. The relative number and basal secretory activity (prolactin/EGF) of these cells were evaluated. Lysis of lactotropes was then determined by using the reverse hemolytic plaque assay. In control conditions, 8.0 ± 0.28 (mean ± SE, n = 3) of all pituitary cells induced plaque formation and plaque area was 1980 ± 230 µm². EGF induced a 50–60% increase in both the proportion of plaque-forming cells and mean plaque area. Frequency distributions of prolactin plaque sizes indicated the existence of two lactotrope populations: small- and large-plaque formers. The additional prolactin cells induced by EGF all formed large plaques. Thus, EGF seems to stimulate prolactin secretion in pituitary cultures by promoting the differentiation of a lactotrope subtype. ST, that supports the growth of other nigral neurons.

621.11

Transgenic MT42 male mice overexpress the human transforming growth factor α (TGF α) in multiple tissues, including the brain. These animals exhibit increased depressive tendencies in the swim test and increased aggressive behavior in the resident-intruder paradigm, when compared with appropriate CD-1 control mice. Brain monoamines revealed that the levels of norepinephrine, dopamine and serotonin (5-HT) were not significantly altered in the hypothalamus, frontal cortex, or brain stem in the male TGF α mice. However, the males showed reduced turnover in the brain stem (p < 0.05). To evaluate the 5-HT turnover, the male TGF α and control mice were treated intraperitoneally with 100 mg/kg tryptophan, a precursor of 5-HT, or with 5-HT uptake inhibitors zimeldine (12.5 mg/kg) or clomipramine (10 mg/kg) 30 min prior to the behavioral tests. Tryptophan did not influence the immobility of the CD-1 mice, but significantly reduced immobility in the swim test in the male TGF α mice (p < 0.01). 5-HT uptake inhibitors significantly reduced aggressive behavior both in the controls and TGF α mice (F(2,4) = 15.3, p < 0.004). These results indicate that the increased depressive and aggressive tendencies noted in the male TGF α mice appear to be reversed by 5-HT uptake inhibitors. Thus, TGF α may interact with 5-HTergic systems in the brain.

621.12

Pregnant albino rats were exposed to vehicle, HAL (5 mg/kg twice daily) or RES (0.2 mg/kg twice daily) over mid gestation (gestation days GD 12-16) or late gestation (GD 18-20). Another control group was pair-fed both to HAL and RES dams. Offspring body weight, regional brain weight, DNA and protein were evaluated in young (GD 20 days age) and adulthood (60-122 days age). Mid-gestational HAL or RES permanently reduced offspring brain weight, regional and whole brain weight and regional brain DNA and protein content at both ages. HAL and RES had comparable effects on whole brain weights (reduction to 90% of control) while RES exposure produced a greater stunting of body weight than did HAL. Comparisons to pair-fed controls revealed that this growth stunting was not due to reduced maternal food intake. Late gestational RES exposure was highly feo-lethal, unlike late HAL exposure. Late gestational exposure to either drug had no effects on offspring brain or body weight that were different from pair-fed controls, with the exception of a permanent and specific HAL not RES stunting of caudate weight, DNA and protein content. In a second experiment, cultured GD 9 embryos were grown for 48 hr in medium containing a range of concentrations of HAL, RES, sulpiride (a specific D2 antagonist) or SCH23390 (a specific D1 antagonist). HAL, substantially reduced embryonic growth at much lower concentrations than did any of the other compounds.

We conclude that prenatal HAL reduces offspring body and brain weight after mid but not late- gestation exposure, through a mechanism which is probably not dopaminergic.

621.13

Hepatocyte-conditioned media enhanced neurite regeneration and their survival from nerve-transplanted terminals of adult rat sciatic nerves with ganglia nerve with ganglia (Norie et al. NeuroReport, 1991). But this media did not promote neurite regeneration or disassociated adult DRG neurons. Application of a hepatocyte secreted factor to adult rat retina explants demonstrated that this factor enhanced neurite regeneration not only in matured peripheral nervous systems but also in adult central nervous systems. In comparison with other known neurotrophic factors, NGF, IGF-I and II, EGF, aFGF, BFGF, Insulin, Interleukin1 to 8 were applied to the peripheral explants. NGF, IGF-I and II, Interleukin 3 and 6 enhanced neurite regeneration, but nerve survival. NGF did not affect neurite regeneration in adult retina explants. These results indicated that the factor secreted from hepatocytes was different from the other known neurotrophic factors. To analyze mechanism of the factor anti-NGF, was administered to the explants. Cerebrospinal fluid extracts of the substantia nigra decreased linearly with age (2-24 months, r = -0.96). In a second experiment extracts of the substantia nigra from adult rats stimulated the growth of low cell density RMT cultures relative to the effects of extracts of the cerebellum (F = 25.7). The stimulatory effect of extracts of the substantia nigra decreased linearly with age (2-24 months, r = -0.96). These data suggest that cells of the substantia nigra produce a soluble factor capable of stimulating the growth of other cells within the nigra. It is possible that the nigral cell loss that accompanies aging and Parkinson's disease may facilitate further neuron loss due to the reduction of this nigral-derived "auto-conditioning" factor.

621.14
DO NIGRAL NEURONS PRODUCE A TROPHIC FACTOR THAT SUPPORTS THE GROWTH OF OTHER NIGRAL NEURONS. NST C. M. Bubel, D., B. Ero, D. C. Sweitzer, J. Vu, and P.M. Carvey. Rush-Presbyterian St. Lukes MC, Chicago IL, 60612

The growth of mesencephalic cultures is density dependent, i.e. with a linear increase in cell number, the number of viable cells with processes increases geometrically. This is often referred to as the "autoconditioning" effect. We performed two series of experiments to determine whether or not this autoconditioning effect was due to a soluble factor. In the first series of experiments, primary, dissociated, E15.5 mid- but not late- gestational exposure, through a mechanism which is probably not dopaminergic.
621.15
DIFFERENCE IN THE ELECTROPHORETIC MOBILITY OF
RAS PROTEIN FROM DIFFERENTIATING AND
PROLIFERATING PC12 SUBCLONES. Y.-Y. Rosenberg, A.
Grimmel* and B.D. Howard. Dept. of Biol. Chem. UCLA Sch. of
Med. Los Angeles, CA 90024
Oncogenic ras changes differentiation of PC12 and proliferation of other cells. A PC12 flat cell variant was transfected with inducible oncogenic ras and subclones were selected. Ras expression caused differentiation of one subclone, 6B3, but it caused proliferation of another subclone, 3A4. Ras protein (p-ras) from 6B3 and 3A4 migrated as 22 kd and 21 kd forms, respectively. After phosphatase treatment, p-ras from 6B3 cells migrated as a 21 kd protein, but the mobility of p-ras from 3A4 cells did not change. This suggests that phosphorylated ras may be involved in differentiation. After treatment of the cells with mevенинов, which inhibits the synthesis of cholesterol precursors and thus protein farnesylation, p-ras from either cell migrates as a 23 kd protein indicating that each is farnesylated. Mevенинов causes PC12 to extend processes that look like neurites. This morphological change is inhibited by introduction into the cells of anti-ras antibody suggesting that ras is involved in this morphological change.

621.17
A NEUROTROPHIC FACTOR OPERATIVE IN THE GENICULOCORTICAL
PATHWAY OF RATS: ISOLATION, PURIFICATION AND IN VITRO TESTING.
K.J. Eplorister, P. Levitt, L. Fischer and T.J. Cunningham. Department of
Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia,
Pa. 19129.
We have previously identified neuron survival promoting activity in present in medium (CM) conditioned by explants of the embryonic primordia of the geniculocortical pathway. The active fraction of the CM promotes the survival of dissociated neocortical (dLGN) neurons following visual cortex lesions in both the neonate and the adult rat. To identify the molecule(s) responsible for this activity, we raised monoclonal antibodies to proteins present in the active fraction. One antibody, 8G6, recognizes a 55kd molecule on Western blots. Following immunofluorphy purification and silver staining of gels, we have detected an additional minor band at 110kd which is diminished upon incubation with DTT, indicating the native protein may be present as a dimer. To assess the neurotrophic effects of this molecule in vitro, dissociated embryonic day 17 lateral thalamic neurons (which include those of the dLGN) were grown with different concentrations of the ptn gene which is expressed in a highly spacially and temporally restricted manner both in vivo and in vitro. Next, stained cells were identified immunocytochemically using an antibody against the 8G6 antigen in developing and in adult rats. Stained cells are particularly prominent in neurons of layer VIB. The cortical white matter and resident glial cells are not stained. Surprisingly, immunopositive cells are rare in thalamo-cortical projection nuclei (including those of the dLGN) which may reflect rapid progression of the factor by responsive neurons. The results suggest that the 8G6 antigen is an endogenous neurotrophic factor in the neonatal rat. Supported by NS16487 from NINCDS and TRG-89-014 from Alzheimer’s Association.

621.19
NEUROTROPHIC ACTIVITY OF OTOCYST-DERIVED FACTOR.
COMPARISON WITH OTHER GROWTH FACTORS AND GANGLIA IN
CLOCK. L.N. Blanch* and C. Coh. Dept. of Anstratomy, SUNY at
Buffalo, Buffalo, N.Y. 14224
During early stages of auditory development, the otocyst releases a factor which promotes outgrowth from the associated statoacoustic ganglia (SAG). Although the otocyst-dervied factor (ODF) promoted outgrowth from the associated statoacoustic ganglia (SAG). Although the otocyst-dervied factor (ODF) promotes outgrowth from the associated statoacoustic ganglia (SAG). Although the otocyst-dervied factor (ODF) promotes outgrowth from the associated statoacoustic ganglia (SAG). Although the otocyst-dervied factor (ODF). Application of ODF promoted outgrowth from explant and the number of surviving cells was less in the presence of these growth factors and thus protein farnesylation, p-ras from either cell migrates as a 23 kd protein indicating that each is farnesylated. Mevенинов causes PC12 to extend processes that look like neurites. This morphological change is inhibited by introduction into the cells of anti-ras antibody suggesting that ras is involved in this morphological change.

621.20
EXPRESSION OF THE PLEIOTROPHIN GENE IN DEVELOPING MOUSE
CENTRAL NERVOUS SYSTEM. H.-J. Yeh, J. Silos-Santiago, R.P.
Gillmaner, Y.-S. Li. D. Snider. and T.F. Deuel*. Dept.
Department of Anatomy and Neurobiology, Medical College of
Expression of the ptn gene during embryogenesis and in maturity.
Expression of the ptn gene during embryogenesis and in maturity.
Expression of the ptn gene during embryogenesis and in maturity.
Expression of the ptn gene during embryogenesis and in maturity.
Expression of the ptn gene during embryogenesis and in maturity.
Expression of the ptn gene during embryogenesis and in maturity.
2.3.5 REDUCTION IN ADJUVANT ARTHRITIS-INDUCED HYPERVENTILATION IN RATS WITH ADRENAL MEDULLARY TRANSPLANTS IN THE SPINAL CORD SUBARACHNOID SPACE

H. T. Tasto, Y. H. Wang, and J. Sagen, Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL 60612

Our previous studies have shown that transplanted adrenal chromaffin cells into the CNS may produce acute analgesic effects. We have now extended this study to determine the reduction in chronic pain by the implants.

The transplants were made by grafting a suspension of bovine chromaffin cells into the subarachnoid space of rats with adjuvant-induced arthritis. The animals were sacrificed after 2 weeks, and the spinal cords were processed for immunofluorescence staining for tyrosine hydroxylase.

Results indicated that the transplanted adrenal chromaffin cells showed a very dense ingrowth of fibers, indicating that the transplants had survived and were functional in the spinal cord. The staining was seen in the spinal cord, indicating that the transplants had successfully integrated into the CNS and were producing catecholamines.

These findings suggest that adrenal medullary transplants may have potential as a treatment for chronic pain syndromes such as arthritis and may provide new avenues for the development of analgesic therapies.
622.7 SODIUM BUTYRATE-TREATED PC12 CELLS AS AN ALTERNATIVE GRAFT SOURCE FOR PAIN REDUCTION. P.H. Kim and J. Sagar*. Dept. of Anat. and Cell Biol. Univ. of Illinois at Chicago, Chicago, IL 60612.

Our lab has previously demonstrated that mature chromaffin cells transplanted into the spinal cord and periaqueductal gray can reduce pain by providing a basal source of opioids and catecholamines. However, large scale application of this approach is limited by the availability of these postmitotic cells. PC12 cells have the added advantages of readily available, reproducibility, and uniformity. PC12 cells, unlike mature aromatic aminergic chromaffin cells, contain very low levels of opioid peptides. The goal of these studies was to produce PC12 cells with increased opioid peptide production for pain reduction. Preliminary evidence in agreement with previous studies showing that sodium butyrate treatment can increase Met-enkephalin levels 2 to 3 fold in PC12 cells (Byrd et al., 1987). However, the Met-enkephalin levels of PC12 cells only returned to baseline after butyrate treatment is stopped. In order to permanently increase Met-enkephalin production, butyrate stimulated PC12 cells were treated with antidepressants like bupropion and bupropion-2'-deoxyribose (BDU), either immediately after butyrate stimulation or four days following withdrawal of butyrate. Both groups showed that butyrate treated for two days, and were then prepared for neurochemical analysis on the eleventh day of the experiment. Preliminary data revealed a 15 to 25 fold increase in Met-enkephalin levels in the first group compared to controls, while the second group only showed a 2 to 3 fold increase compared to controls. In addition, the antidepressant treatments were successful in inhibiting PC12 cell proliferation. The data suggests both synergistic and perhaps independent action of antidepressive agents with butyrate on increasing Met-enkephalin production. Preliminary findings have revealed that PC12 cells transplanted into the spinal cord and PAG showed inhibited tumor growth and good survivability. These results suggest that butyrate-treated PC12 cells may provide an alternative donor source for pain reduction. (Supported by NHLBI grant NS25604.)


Fetal transplants have been promoted to at least partial recovery of a variety of motor behaviors after intraspinal grafting procedures in injured adult animals (Stokes et al., 1990). In spite of these promising findings, certain spontaneous behavioral paradigms (open-field, inclined plane and grid-walking analysis) often reveal early deficits in transplanted animals when compared to injured controls. We have also previously associated graft integration and potential behavioral effects with neovascularization of the graft segment. In the present experiments, we have investigated whether suspension transplants placed concomitantly with a potent angiogenic agent (nimodipine) can improve these early alterations and diminish the differences in final outcome scores. Rats were pretrained behaviorally, anesthetized and injured with an electro-mechanical impactor device. Ten days later they received an intraspinal transplant of dissociated E14 spinal cord cells. In addition, 2 (10 mg) slow-release tablets impregnated with nimodipine were placed (S.O) in order to enhance the angiogenic process. Behavioral testing was conducted weekly for 2 months and the success of angiogenic procedures evaluated by stereological analysis of semi-thin plastic sections of tissues in and around the graft site. Graft capillarity (33% increase in surface fraction), mean vascular diameter and graft size were all enhanced by nimodipine. Integration of graft tissue with the adjacent host was excellent and grafts often included large fascicles of preserved myelinated fibers. Previous described differences in behavioral outcomes after grafting were not seen in the nimodipine treated animals. Angiogenesis may therefore be an important factor in graft development and behavioral outcomes. Supported by NS-10165 and NS-27511.

622.10 STRATEGIES IN TRANSPLANT THERAPY FOR SPINAL CORD INJURY. Claire E. Hulsebosch and John Dorman. Dept. Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, TX 77555.

Spinal cord injury results in a devastating loss of behavior below the level of the lesion. In a typical lesion, several tracts are severed, grey matter is destroyed and nearby mildly injured and uninjured neurons may be able to grow neurites a few mm., but no further. In the presence of exogenous therapy, the abortive growth may be encouraged. Examples of therapy to encourage growth include the use of one or more of the following: peripheral nerve transplants, antibodies against inhibitory substances, antibodies to neurotrophins, neurotrophins, and the use of transplanted cells (both embryonic and adult tissues). The role of neural culture in stimulating growth within the spinal cord after lesions which include the corticospinal tract. Technical issues will be presented to insure success. In addition, progress in our laboratory on other strategies for spinal cord repair will be presented. Supported by NIH Grants NS 11255, NS 01217, Bristol-Squibb Myers.


Male (n = 52) and female (n = 60) guinea pigs were maintained on long days (16L:8D) from 0.5 - 19 months. Their visual acuity was determined by recording spatial-frequency tuning curves of the pattern electroretinogram that was generated in response to gratings stimuli. The thresholds resulted in continuous oscillation by the females from sexual maturity until death. Both males and females showed systematic changes in acuity throughout the life span. Male acuity is higher, thereafter it declined slowly. Female acuity was not significantly different from that of the males at the youngest ages. In contrast to the slow, steady decline of the males, female acuity rapidly rose to a peak at 5-12 months; thereafter it declined rapidly and reached the male level at 18-19 months. Preliminary findings with female guinea pigs maintained under short day length (8L:16D), which inhibits ovulation, indicated an improvement in acuity. The biphase age-acuity curve of female guinea pigs is similar to the human age-acuity curve, which is also biphase. Both of these curves reach a peak at approximately 10% of life span. These results suggest that in females, the endocrine system plays a role in visual aging and that female guinea pigs are a useful model for some aspects of human visual aging.


Fischer 344 (F344) rats are reported to suffer from an age-related retinal degeneration, but little data are available on the exact gender, regional, and age effects on peripheral degeneration. The long-term objective of this study is to develop strategies for peripheral degeneration. To determine whether the retinal degeneration in the F344 rat is affected by the combination of gender, regional, and age-related factors. (Supported by NEI-05262, and the Rochester Eye Bank)
623.3 EFFECTS OF SIZE OF ATTENTIONAL FOCUS ON VISUAL SEARCH IN AGED ADULT RATS. THOMAS D. WOODWARD, FREDERICK E. GREENWOOD, BACLINGA PANICKER and J.V. HASKO. Catholic University of America, Washington,DC and NIA, Bethesda, MD, 20004.

Feature-based theory (Treisman & Gelade, 1980) claims that conjunctive search requires focal attention directed serially over the display, while feature search depends on parallel processing. Plude & Duossard-Rosemond (1989) reported that older adults are disproportionately slowed on conjunctive but not feature search. Greenwood, Parasuraman and May (1992) reported that engaging visuospatial attention in response to valid cues is impaired in normal aging, while disengaging attention from invalidly cued locations is slowed with age. Now we ask whether (a) age alters the ability to adjust the size of the attentional focus and (b) varying the precision of location cues counteracts the effects of age on conjunctive search. Forty old and young Ss searched for both features and conjunctions of features over 10 or 15 display elements comprised of three colors and three letters. Cues preceded targets by 500 msec and were rectangles drawn around either the target letter, column containing the target or side of the screen containing the target (valid) or another letter, column or side (invalid). Increasing display size slowed the old more than the young in conjunctive search but not in feature search. Cue size also had little effect on feature search. In conjunctive search RT decreased linearly with decreases in cue size but similarly in young and old. Cue validity effects were largest in conjunctive search at the smallest cue size with old Ss showing greatest effects. Results indicate (a) old Ss are as adept as young in manipulating size of attentional focus, (b) old Ss are slower than young to shift attention during serial search even with valid cues, and (c) serial but not parallel search is dependent on attentional focus.


Representative groups of the adult life span of male Fischer 344 (F344; 7,13,23 mo), Brown Norway (BN; 7,13,23 mo) and F344/BN F1 (F1; 7,13,23 , mo) rats were tested in a battery of behavioral testing including 15-min and 24-hr spontaneous activity (SA), inclined screen (IS), wire hang (WH), and NIA, Bethesda, MD, 20064.

623.5 SEVERITY OF SPATIAL LEARNING IMPAIRMENT IN AGING ASSESSED BY THE DEVELOPMENT OF A LEARNING INDEX. Rebecca D. Barwell*, Margaret Burchinal, and Michela Gallagher. Dept. of Psychology, Univ. North Carolina, Chapel Hill, NC 27599.

The Morris spatial learning task has become widely used in neurobiological studies and in the characterization of cognitive decline in the aged rat. An important insight in the study of aging is that individual differences in spatial learning impairment are often good predictors of neurobiological age. Thus behavioral characterization can provide an important background for studies of brain aging.

This report describes new methods for analyzing performance in the Morris task. For the development of a learning index that reflects the accuracy of spatial learning, the animal's proximity to the target location of the escape platform was used. This proximity measure was integrated over the course of training using a set of weights based on the learning curve for young rats (N=70). Data from aged rats (N=98) indicated that learning index scores differed for subpopulations of aged rats (impaired and unimpaired) based on performance in a Morris water maze. Total RNA was isolated from the medial septum and hippocampus in both young and old rats. The mRNA for BAPP increased in the hippocampus of impaired aged rats while unimpaired aged rats did not perform as well as young rats in maze learning. Supported by NIA grant PO1 AG09973 and a NIMH RSDA to MG (K02-MH00406).

623.6 MEASURING CHANGES IN TRANSCRIPT LEVELS FOR VARIOUS GENES IN THE MEDIAL SEPTUM AND HIPPOCAMPUS OF RATS DUE TO AGE AND MEMORY IMPAIRMENT. Rhonda M. Greene, Michael D. Robbins, Michelle Gallagher, and Michael McKinnery. Mayo Clinic, Jacksonville, Jacksonville, FL 32224 and University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

Sixty-six rats were grouped according to age and spatial learning impairment (young, unimpaired aged, and impaired aged) based on performance in a Morris water maze. Total RNA was isolated from the medial septum and hippocampus in both young and old rats. The mRNA for BAPP increased in the hippocampus of impaired aged rats while unimpaired aged rats did not perform as well as young rats in maze learning. Supported by NIA grant PO1 AG09973 and a NIMH RSDA to MG (K02-MH00406).

623.7 EFFECTS OF AGING IN BEHAVIORALLY CHARACTERIZED RATS ON MUSCARINIC RECEPTOR SITES USING IN VITRO AUTORADIOGRAPHY. T. M. Gill*, M. McKinnery, and M. Gallagher. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599 and Mayo Clinic, Jacksonville, FL 32224.

Young (4 mo, N = 8) and aged (25 mo, N = 15) male Long-Evans rats were behaviorally characterized by spatial learning in the Morris water maze. These same subjects were then used to study hippocampal muscarinic binding with quantitative in vitro autoradiography. Quinuclidinyl benzilate (QNB) alone and [3H]QNB in the presence of the M1 receptor antagonist, pirenzepine, were used to determine total muscarinic, M1, and non-M1 binding.

No age differences in total, non-M1, or M1 binding were found throughout the hippocampus. However, analysis of covariance for M1 sites in ventral dentate gyrus revealed that subgroups differed significantly when binding was assessed in relation to learning: high binding was associated with better learning in aged rats with preserved spatial abilities (r values range from -0.70 to -0.89), but opposite correlations were found for the young and aged impaired subgroups (r values range from -0.50 to 0.60). Further examination of these autoradiographs showed that age-related changes in muscarinic sites are evident in other areas (i.e. frontal cortex and medial septum).

Supported by NIA grant PO1 AG09973 and a NIMH RSDA to MG (K02-MH00406).

623.8 AGE-RELATED CHANGES IN MUSCARINIC CHOLINERGIC SUBTYPES IN RAT BASAL FOREBRAIN AND FRONTAL CORTEX. Robert P. "Yapugo", Sean A. Gallego, Jason S. Weisstein, and Barry B. Wolfe. Department of Pharmacology, Georgetown University School of Medicine, 3900 Reservoir Road, Washington, D.C. 20007.

Muscarinic cholinergic receptor subtypes were examined in young (4-5 months) and aged (24-25 months) Long-Evans rats. The aged rats were further divided into two groups: 1) Aged Unimpaired rats that had similar Morris water maze performance to that of young rats, and 2) Aged Impaired rats that did not perform as well as young rats in the Morris water maze. Antisera selective for the m1-m4 receptor subtypes were used to examine differences in receptor density in these three groups of rats in the basal forebrain and frontal cortex (Mol. Pharmacol., 39: 643-649, 1991; Mol. Pharmacol., 40: 28-35, 1991; Mol. Pharmacol., 40: 783-789, 1991; Soc. Neurosci. Abstr., 17, 1532, 1991). There were no differences in any receptor subtypes between the aged impaired and the aged unimpaired rats in either brain region. However, there was significant decrease in at least the m4 receptor subtype in aged animals compared to young in the basal forebrain (young, 0.81 ± 0.07; aged unimpaired, 0.599 ± 0.046; p < 0.01; aged impaired, 0.382 ± 0.036 pmoles/mg protein, p < 0.01; N=8). These decreases in m4 receptor were not observed in the frontal cortex (young, 0.380 ± 0.027; aged unimpaired, 0.417 ± 0.007, p<0.05; p0.05; p<0.01). Supported by AG09973 and AG09884.

The purpose of this investigation was to examine the expression of the five muscarinic receptor mRNAs in three rat brain regions and to determine if there are age-related differences in the expression of these transcripts. Poly (A)* RNA from neostriatum and cortex from Fischer 344 X Brown Norway hybrid rats (3, 18 and 33 months) were analyzed by Northern blotting with 32P-labeled oligonucleotide probes of m1, m2, m3, m4 and m5 receptors. Messenger RNA levels were quantitated by densitometry. In the neostriatum, the m2 mRNA was detected in both groups and was not detected in cortex. In the cortex the m1 mRNA was detected in the 3 month old group and was not detected in the 18 and 33 month old groups.

The relative regional abundance of each of the five mRNAs was similar in all age-groups. The m1, m2 and m3 mRNAs were more abundant in cortex and hippocampus than in neostriatum, the difference being more pronounced with m3 mRNA. The m4 mRNA was most abundant in the neostriatum. The m5 mRNA was most prominent in the hippocampus, weakly detected in neostriatum, and not detected in cortex. With age the expression of m5 mRNA in the neostriatum decreased. No significant age-related changes in the expression of other receptor mRNAs were observed. How this finding relates to other well documented changes in neostriatum with aging remains to be determined. (Supported by a grant from American Federation for Aging Research).

ATTENUATION OF TYROSINE HYDROXYLASE ACTIVATION FOLLOWING NEUROTOXIC INSULT TO THE HIPPOCAMPUS OF AGING RATS. J.R. Unemoto*, Dept. of Anatomy and Cell Biology, Univ. of Ill. at Chicago, Chicago, IL 60680.

The locus coeruleus (LC) loses up to 50% of its neurons during normal aging. However, no differences, or possibly, increases in static measures of synaptic integrity, such as norepinephrine content or tyrosine hydroxylase (TH) activity, have been reported in brain regions innervated by LC neurons in aged vs young animals. Yet, behavioral deficits associated with attenuation of LC function seen in aged animals suggests that this neural system has lost its ability to rapidly respond to stimuli or insult. In these preliminary studies, the neurochemical adaptability of LC neurons in aging rats has been assessed using a paradigm originally reported by Aschen and Zigmond (J. Neurosci. 1:493, 1981). Using this model, TH activity was assessed in the hippocampus of young (2 month) and old (24 month) Fisher-344 male rats 72 hours following the infusion of 200 µg of the neurotoxin 6-hydroxydopamine (TH) vehicle into the lateral ventricle. Interestingly, it was noted that baseline TH activity measured under optimal conditions (pH=6, 0.3 mM 6-MPH, 30 µM tyrosine) was twofold higher in unlesioned old animals compared to the young animals (~12 vs 6 pmol/min/mg protein). The lesion resulted in a 55-59% decrease of TH activity measured under optimal conditions. When measured under suboptimal conditions (pH=6.5, 0.7 mM 6-MPH, 30 µM tyrosine), TH activity in young lesioned animals was 83% of that measured in young vehicle treated animals. However, in the old lesioned animals, TH activity measured under suboptimal conditions was only 40% of that measured in age-matched control animals. These data suggest that the ability of LC neurons to rapidly respond and compensate to this insult is attenuated in the old animals due to a deficit in this system's capacity to activate TH. (NIA grant AG09587)


Although synaptic densities are deficient in the retention of LTP, they potentiate to the same extent as young ones (Barnes, J. Comp. Physiol. Psychol., 1978, 45, 74; deToledo-Morrell et al., Neurobiol. Aging, 1986, 7, 561). We showed earlier (Genismin et al., Brain Res., 1991, 566:77) that the induction of LTP in young rats is followed by a selective increase in the number of axospinous synapses per neuron (ac) after a single frequency stimulation. The aim of the present study was to determine if old potentiated animals exhibit the same structural synaptic modification. Aged F344 rats (27 mo. old) were implanted with stimulating electrodes in the medial perforant path and recording electrodes in the hilus of the ipsilateral dentate gyrus. Potentiated animals were stimulated with 150 µA at a stimulation frequency of 50 Hz for 1 hour. Control animals were implanted with the same electrodes and were stimulated with only 50 Hz for 1 hour. The number of axospinous synapses per neuron was estimated for various synaptic subtypes in the middle (MML) and inner molecular layer of the dentate gyrus using the disector technique. Only axospinous synapses with a segmentated PSD were significantly increased in numbers in the MML of aged rats relative to their stimulated (at a frequency of 0.2 Hz) or unstimulated (control) counterparts, a modification that is associated with a prolonged, dihydropyridine-sensitive Ca current.

DENDRITIC GROWTH IN ADULT GRANULE NEURONS IN THE RAT DENTATE GYRUS REVEALED BY THE UNBIASED DISECTOR TECHNIQUE. F. Moret et al., G. Genismin, L. deToledo-Morrell, J. W. Landfield, Dept. of Pharmacology, The University of Chicago, Chicago, IL 60637.

During late postnatal development (14 to 60 days of age), granule cells in the dentate gyrus lose dendritic branches while their remaining branches continue to elongate to accommodate the molecular layer. Synaptic events associated with growth and regression lead to a conservation of total dendritic length (Rihn & Landfield, Soc. Neurosci. Abstr., 91). Using single electrode voltage clamp, several types of voltage-activated Ca currents were measured in young neurons in the dentate gyrus. In this study, therefore, we tested the response of aged neurons to intracellular application of diethylb cyclic AMP (deAMP) (Thibault et al., Soc. Neurosci. Abstr., 91). Using single electrode voltage clamp, several types of voltage-activated Ca currents were identified, including an L-type current that is associated with a prolonged, dihydropyridine-sensitive Ca current.

Hippocampal CA1 neurons from aged rats also exhibit increased voltage-sensitive Ca spikes and Ca currents. This aging-dependent enhancement of Ca current resembles the deAMP Ca current in young neurons. In this study, therefore, we tested the response of aged neurons to intracellular application of deAMP. We recorded Ca currents from young (3-5 months) and aged (25-27 months) rat neurons treated with TTX and TEA and impaled with pipettes filled with 0.5 M CsCl, 1 mM Mg-ATP, and ± 1 mM deAMP.

In young neurons deAMP again significantly increased the Ca current, whereas this treatment did not influence the aftercurrent in aged neurons (which already show an increased aftercurrent). Neither activation/ inactivation ranges, nor calcium-dependent inactivation were affected by administration of deAMP in young or aged neurons.

Since the effects of aging and deAMP were not additive, the results suggest that these two processes may operate on a common pathway, and that the effects of aging on increased Ca currents may be mediated at least, in part, by increased cAMP-dependent phosphorylation. (Supported by AG 04547 and Miles Inc.)


The present study was designed to reexamine the currently accepted view that synapses in the dentate gyrus are lost with age, but are equally potentiated and maintained. The number of axospinous synapses per neuron (ac) was estimated, with use of the unbiased disector technique, in young (420 ± 67 um) and aged (566 ± 77) rats. The number of axospinous synapses per neuron (ac) was greater in young rats (140 ± 20) than in aged rats (80 ± 10). Significant changes were observed in the number of axospinous synapses per neuron (ac) in the middle molecular layer (MML) and inner molecular layer (IML). In young rats, the number of axospinous synapses was significantly higher in the MML (by 23.6%) and IML (by 22.2%) of aged rats relative to young adults (p < 0.005 in both cases). These results suggest that the number of axospinous synapses per neuron is significantly diminished in the MML (by 23.6%) and IML (by 22.2%) of aged rats relative to young adults (p < 0.005 in both cases). These data suggest that the number of axospinous synapses per neuron is significantly diminished in the MML (by 23.6%) and IML (by 22.2%) of aged rats relative to young adults (p < 0.005 in both cases). These data suggest that the number of axospinous synapses per neuron is significantly diminished in the MML (by 23.6%) and IML (by 22.2%) of aged rats relative to young adults (p < 0.005 in both cases).

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623.15


Entorhinal cortex lesioning (ECL) destroys a major hippocampal input and leads to axonal sprouting in the dentate gyms. Glucocorticoids are known to inhibit this reorganization process. In the present study, we examined hippocampal glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA expression using in situ hybridization following unilateral ECL in the rat. Film autoradiography showed that as early as one day post lesion, a dramatic bilateral decrease in GR mRNA was observed in the granular dentate gyms. By contrast, in the CA1 region, a moderate increase in GR mRNA was detected. GR mRNA levels in both regions returned to those of control animals 2 days post lesion indicating that these effects were transient. Adjacent sections hybridized with probes to MR mRNA revealed no apparent changes in expression in any hippocampal sub-regions as a result of ECL. Western Blots from total hippocampus homogenates, using the BeGR2 antibody (Affinity Bioreagents), confirmed an overall decrease in GR protein in the hippocampus. This decrease was also transient with maximal effect being observed 2 days post lesion and levels returning to control values 4 days post surgery. These results suggest that the regulation of GR but not of MR is associated with synaptic reorganization. Interestingly, the opposing changes in GR mRNA expression seen in the dentate gyms and CA1 following ECL may be related to the pronounced vulnerability of the CA1 cell field and relative resistance of the dentate gyms to a number of conditions that threaten neuron survival.

623.16


Entorhinal cortex lesioning (ECL) destroys a major hippocampal input and leads to axonal sprouting in the dentate gyms. Glucocorticoids are known to inhibit this reorganization process. In the present study, we examined hippocampal glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) mRNA expression using in situ hybridization following unilateral ECL in the rat. Film autoradiography showed that as early as one day post lesion, a dramatic bilateral decrease in GR mRNA was observed in the granular dentate gyms. By contrast, in the CA1 region, a moderate increase in GR mRNA was detected. GR mRNA levels in both regions returned to those of control animals 2 days post lesion indicating that these effects were transient. Adjacent sections hybridized with probes to MR mRNA revealed no apparent changes in expression in any hippocampal sub-regions as a result of ECL. Western Blots from total hippocampus homogenates, using the BeGR2 antibody (Affinity Bioreagents), confirmed an overall decrease in GR protein in the hippocampus. This decrease was also transient with maximal effect being observed 2 days post lesion and levels returning to control values 4 days post surgery. These results suggest that the regulation of GR but not of MR is associated with synaptic reorganization. Interestingly, the opposing changes in GR mRNA expression seen in the dentate gyms and CA1 following ECL may be related to the pronounced vulnerability of the CA1 cell field and relative resistance of the dentate gyms to a number of conditions that threaten neuron survival.

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624.3


Previous reports have shown that there are age-related reductions in muscarinic receptor (mACHr) sensitivity to agonist stimulation. The alterations are the result of specific changes in mACHr-G protein interactions [expressed by increased stimulated low K+ (0-20mm) GTPase activity (an indicator of receptor/G protein coupling/uncoupling) in hippocampus (HIP) and olfactory bulbs (OB)]. Our results show that reductions in phosphoinositide (PI)-mediated signal transduction (PI) are not age-related. Present experiments assessed possible age- and disease-related reductions in C-SLKM GTPase activity in young (Y), Alzheimer's disease (AD), and basal ganglia (BG). Results showed that while there were age- and disease-related reductions in C-SLKM GTPase activity in BG (F(4,40) = 7.05 p < 0.01), (e.g., 10^4 carb. df = 40 t's =: Y vs AM 2.71, p < 0.01; AM vs AD 2.22, p < 0.05; Y vs AD 4.93, p < 0.001), only age- and not AD-related deficits were observed in HIP (e.g., t (6) Y vs AD = 1.08, p > 0.05; t (7) Y vs AM = 2.58 p < 0.05). Results suggest there are age- and disease-related changes in mACHr signaling that may contribute to reduced PI-mediated ST. Moreover, there could also be some compensatory alterations in AD HIP in C-SLKM GTPase activity. Additional experiments examining C-SLKM GTPase activity in HIP and CPU from infected rats have shown reductions in this parameter as well as ST deficits, suggesting free radical involvement in these deficits.

624.4

AGE-RELATED CHANGES IN THE RAT STRIATUM: A MICRODIALYSIS STUDY. R.B. Maloney* and G.A. Gerhardt, Dept. of Psychiatry & Psychology, University of Colorado Health Sciences Center, Denver, CO 80262.

In vivo electrophysiological experiments have shown that extracellular dopamine (DA) in the rat striatum following K+ stimulation is reduced in aged rats as compared to young rats. In this study, the technique of in vivo microdialysis was used to further investigate both basal and K+ evoked overflow of DA in the striatum of young and aged rat. Eleven Fischer 344 rats were used (n = 5: 24-26 mos, n = 6: 2-5 mos). Four mm long, 300 µm diameter dialysis probes were stereotaxically implanted into the rat striatum (+1.65 mm A.P., ±1.65 mm Lat. with respect to bregma, Paxinos & Watson). Probes were perfused with artificial CSF at a flow rate of 1.2 µl/min. Samples were taken every 10 mins and analyzed by HPLC with electrochemical detection. When the variability of 5 consecutive DA sample peak heights was < 10%, the perfusion medium was changed to 100 mM K+ CSF for 10 mins. The K+ stimulation produced a significant difference in the percent increase for DA, 4119.27% in aged rats and 1509.33% in young rats. Aged rats had significantly lower baseline amounts of DA, DOPAC and HVA. In addition aged animals had significantly lower levels of DOPAC following K+ evoked DA release. (Supported by USPHS Grants AG06434, AG00441 and NS-0199)

624.5

AGE-RELATED CHANGES IN GLUTAMATE EVOKED DOPAMINE OVERFLOW IN THE STRIATUM OF THE FISCHER 344 RAT. M.N. Friedemann and G.A. Gerhardt, Dept. of Pharmacology and Psychiatry, University of Colorado Health Sciences Center, Denver, CO 80262.

Glutamate and other excitatory amino acid agonists can elicit dopamine (DA) release from nerve terminals in the striatum. Age-related alterations in this interaction between corticostriatal and nigrostriatal inputs may contribute to motor deficits commonly seen during aging in mammals. The purpose of this study was to examine the effects of aging on glutamate-evoked overflow of DA in the striatum of Fischer 344 rats. Male F344 rats that were 6 or 24-months old, were anesthetized with urethane and placed in a stereotaxic apparatus. L-glutamate (0.5 to 3 mmol) was ejected in situ from glass micropipettes 300 µm away from a Nafton-coated carbon fiber electrode. DA concentrations were measured in real time using an electrochemical recording system (IVEC-5; Medical Systems, Inc.). The results show that the average amplitude of DA signals was significantly lower in aged rats (0.67 ± 0.09 µmol vs. 1.35 ± 0.14 µmol; p < 0.01). However, the doses of glutamate applied were significantly greater in the aged rats (1.2 ± 0.1 mmol vs. 0.9 ± 0.1 mmol; p < 0.01). Although this finding may be explained by an increase in uptake of glutamate from the extracellular space, these results suggest that there may be age-related changes in sensitivity to the effects of glutamate on DA nerve endings, in that higher doses of glutamate produced lower amplitude signals in aged rats.

624.7

AGE-RELATED VARIATIONS IN THE STEADY STATE LEVELS OF ALTERNATIVELY SPliced D2 RECEPTOR mRNAs IN BRAIN AREAS OF DIFFERENT RAT STRAINS. N. Brunello, F. Pujol, M. Marzio, M. Fasano, A. Ragonese, M. Giorgi*, Center of Neuropharmacology, Institute of Pharmacological Sciences, University of Milan, Via Balzaretti 9, 20133 Milan and Facoltà di Economia, UNIMIB, Milano, Italy.

Dopamine D2 receptor gene produces two receptor isoforms by alternative RNA splicing, the so called D2-L and D2-S subtypes. Age-related reduction of D2 receptor density in various brain areas, particularly in the striatum, have been reported by means of radioligand binding techniques. Using the highly sensitive reverse transcription-polymerase chain reaction analysis we have investigated possible changes in the relative abundance of the mRNAs encoded by the dopamine receptor subtypes D2-L and D2-S in brain areas of aged rats. We have observed age-related decrease in the D2-L mRNA ratio in the striatum and in the hippocampus of aged rats, whereas no significant differences were observed in the nucleus accumbens and in the frontal cortex.

624.8


Olfactory function is compromised in humans as a result of normal aging and in neurodegenerative disorders. To begin a biochemical characterization of the aging process in a rodent olfactory model, we tested the hypothesis that age-related alterations occur in the expression of tyrosine hydroxylase (TH), the first enzyme in the dopamine biosynthetic pathway. Previous experiments in young animals demonstrated that TH expression in olfactory nerve cells, the main olfactory bulb is significantly reduced following exposure to nerve terminal inactivation. The decrease in mRNA levels in aged nuclei is greater than the cell loss, and prompts the question of whether the surviving neurons are synthesizing less D2 receptor mRNA.

Previous studies were directed toward determining the relative rates of synthesis for dopamine D2 receptor mRNA in young and aged rats. In our experiments in young animals demonstrated that TH expression in periglomerular cells of the main olfactory bulb is significantly reduced following exposure to nerve terminal inactivation. The decrease in mRNA levels in aged nuclei is greater than the cell loss, and prompts the question of whether the surviving neurons are synthesizing less D2 receptor mRNA.

The D2 mRNA was synthesized at a decreased rate in the aged nuclei (57%, p<0.01). There was no significant difference between young and aged nuclei in tubulin or D2 mRNA as detected on the blots. The decrease in D2 mRNA synthesis is partly attributed to the loss of D2 cells, but also seems to reflect an age-dependent effect on surviving D2 neurons.

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During aging, dopaminergic neurons synthesize and secrete less dopamine (DA) as demonstrated by the observation that the rate of secretion of DA into hypophyseal portal blood is significantly less in aged rats than in young rats. The in vitro activity of tyrosine hydroxylase (TH), the rate-limiting enzyme of catecholamine (CA) synthesis, is regulated by a variety of factors, such as hormones and various protein kinases, including cyclic adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase (PKA). This study was conducted to examine the role of the PKA pathway in the regulation of the in situ molecular activity of TH in the brains of aged and young control female rats. Hypothalamus and corpus striatum (CS) were incubated for 60 min with various agents that modify the PKA pathway. The incubation mixture contained 10^-4 M NSD 1015 [dihydroxyphenylalanine (DOPA) decarboxylase inhibitor]. At the end of incubation, the tissue was homogenized and DOPA was measured by HPLC with electrochemical detection and TH by immunoblot electrophoresis analysis. Forskolin, an activator of adenylyl cyclase, at concentrations of 1, 5, and 25 µM significantly (P<0.01) increased the TH activity in tissues from both age groups. Theophylline (1 mM, phosphodiesterase inhibitor) did not affect TH activity in the hypothalamus of aged and young rats. Ca^2+ (2-3 mM) did not change with age regardless of calcium concentration, suggesting that the function of Ca^2+ in the hypothalamus and CS of young and aged rats. In addition, specific cAMP agonist, (3)-cyclic adenosine 3',5'-monophosphothioate, significantly (P<0.001) increased the TH activity in tissues from both age groups. cAMP agonists (50 µM) of various adrenergic receptors tail arteries from 6 and 20 month old rats were stimulated with short stimulation trains in the presence of 3 different concentrations of calcium (460 V, 1 msec). During aging, dopaminergic neurons in aged rats may involve reduced synthesis of cAMP.


Activity-dependent modifications of glutamatergic synapses are likely to be involved in learning processes. Learning deficits are particularly frequent with age and have been postulated to be due to a decrease in NMDA receptor responsiveness. We investigated possible changes in glutamate receptors with aging using quantitative ligand binding autoradiography. Tritiated AMPA and CNQX on one hand and TCP on the other hand were used to label the NMDA and NMDA receptors respectively. Two groups of Fisher (F344) rats, 2-3 and 29 months old were sacrificed, their brains rapidly removed and frozen. Ligand binding autoradiography was then performed on 10 um thick sections. The aged animals exhibited a reduced binding for both AMPA and CNQX throughout the brain while TCP binding remained unchanged. The decreased binding was particularly marked in the hippocampus. These results suggest that the number of AMPA receptors is decreased with age while the number of NMDA receptors remains unchanged. As AMPA receptor properties have been implicated in learning-induced synaptic plasticity, a decreased number of receptors might account for learning deficits observed in aged rats.

ADVANCING AGE DOES NOT AFFECT α2 ADRENERGIC RECEPTOR FUNCTION ON BINDING IN F-344 RATS. J. BUCHELOV* and S.P. DUCKLES. Pharmacology. Coll. of Medicine, Univ. of California, Irvine, CA. 92717.

Fractional norepinephrine (NE) release evoked by long stimulation trains significantly decreases 20 months of age in tail arteries of F-344 rats; however the maximal effect of idazoxan to increase NE release does not change with age. To examine further the effect of age on the overall function of α2-adrenergic receptors tail arteries from 6 and 20 month old rats were stimulated with short stimulation trains in the presence of 3 different concentrations of calcium for 4 sec at 8 Hz (60 V, 1 msec). Perfusion from 5 trains was pooled. Tail arteries were treated with 10^-7 M deoxytocosterone and cocaine and NE release measured by HPLC with electrochemical detection. The ability of idazoxan to increase NE release did not change with age regardless of calcium concentration, suggesting that the function of α2-adrenergic receptors in the tail artery remains unchanged with age. Binding studies in kidney and brain homogenates were performed with [3H]idazoxan. Idazoxan dissociation constant (Kd) and maximal binding (Bmax) did not change with age, suggesting that there are no age-related changes in the affinity or number of α2-adrenergic receptors in the kidney or brain. For these reasons, we considered that the differences in the effect of adrenergic agonists on NE release cannot account for the age-related changes in NE release. Binding studies in kidney and brain homogenates were performed with [3H]idazoxan. Idazoxan dissociation constant (Kd) and maximal binding (Bmax) did not change with age, suggesting that there are no age-related changes in the affinity or number of α2-adrenergic receptors in the kidney or brain. Presumably, these results indicate that in the in vitro system, aldosterone (ALD) increased the NE release in both age groups. This result indicates that the PKA pathway modulates TH activity in the hypothalamus of young and aged rats. Impairment of hypothalamic dopaminergic neurons in aged rats may involve reduced synthesis of cAMP.

EFFECT OF GOLD-THIO-GLUCOSE (GTG) ON HYPOTHALAMIC OXYTOCIN RECEPTORS AND NEUROPEPTIDE Y (NPY) mRNA BY FASTING DURING AGING IN MALE RATS. C.V. Mobbs* and S.P. Kleopoulos. Regulation of glucose metabolism is impaired during aging, elevated glucose can accelerate age-correlated impairments, and dietary restriction delays some impairments. NPY mRNA in the arcuate nucleus appears to play a role in regulating glucose metabolism and is induced by fasting. We therefore examined if the induction of NPY mRNA by fasting is impaired during aging. 6-month-old, 12-month-old, and 18-month-old male Sprague-Dawley rats were housed for 72 hours, then sacrificed at 1900 h, one hour after lights went out. Control rats were housed for 8 h, allowed to drink milk at 1800 h and sacrificed at 1900 h. Brains were fresh-frozen in dry ice, then cut into 6 micrometers sections for analysis by in situ hybridization. Single-stranded neuropeptide Y cDNA probes were labelled with tritium by asymmetric PCR (using a plasmid generously supplied by S. Sabol). Sections were fixed for 3 weeks, then dipped in emulsion and exposed for another 3 weeks. Quantification of both film and emulsion indicated that in 6-month-old rats NPY mRNA was significantly induced by fasting, but in 12- and 18-month-old rats the induction was not significant. These data suggest that in aging rats regulation of hypothalamic NPY mRNA is impaired. Supported by the American Diabetes Association and a Long Island Heart Foundation Equipment Grant.

Reproductive senescence in female rodents is characterized by impairments in estrogen-regulated neuroendocrine functions. Some of these age-correlated impairments appear to be due to persistent deleterious effects of estrogen. We therefore examined if hypothalamic oxytocin receptors and lordosis behavior, both of which are induced by estrogen, exhibit persistent effects of estrogen during aging. 3- and 10-month-old cycling rats, and 15-month-old non-cycling rats were ovarioctomized. Ten days after ovarioctomy, rats were given silastic capsules containing 5% estradiol, or empty sham implants (n=6/group), and sacrificed four days later. Estradiol (E2) significantly increased both oxytocin receptors (assessed by in vitro autoradiography using 125I-ornithine vasotocin) and lordosis reflex (assessed by daily manual stimulation) in all age groups; conversely, E2 decreased plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH). In the sham-implanted groups, 2 weeks after ovarioctomy, oxytocin receptors and lordosis reflex increased with age, and LH and FSH decreased. These data suggest either that a non-ovarian source of E2 increases with age, or that there is a persistent effect of E2 on several neuroendocrine functions during aging.

Supported by the American Federation for Aging Research.

FOOD RESTRICTION SUPPRESSES HYPOTHALAMIC PROOPiomelanocortin (POMC) MESSENGER RNA THROUGHOUT THE LIFESPAN OF THE MALE FISHER 344 RAT. J.P. Nelson* and K. Kurella, Dept. of Physiology, University of Texas Health Science Center, San Antonio, TX 78284.

POMC is synthesized in the arcuate nucleus of the hypothalamus (HYPO) and is the precursor of several neuropeptides. HYPO POMC mRNA is suppressed in food restricted rats, it was of interest to determine if POMC mRNA is suppressed in food restricted rats. We also sought to determine if HYPO POMC mRNA declines with age in the Fisher 344 rat, as reported for other strains of rat and mouse. Rats were food restricted (FR) to 60% of ad libitum fed rats, it was of interest to determine if POMC mRNA is suppressed in food restricted rats. We also sought to determine if HYPO POMC mRNA declines with age in the Fisher 344 rat, as reported for other strains of rat and mouse. Rats were food restricted (FR) to 60% of ad libitum fed rats, and were killed between 0800 and 1200 (lights on: 0400 h) at 6, 12, 18 and 24 months of age. HYPO were dissected, snap frozen, and RNA was extracted by the guanidinium cesium chloride method. POMC mRNA was measured by solution hybridization/RNAse protection using a homologous 32P-cRNA probe complementary to a portion of rat POMC mRNA. At all ages, POMC mRNA levels were 20-40% lower in FR than in AL rats (p<0.05). There was no age-related change in POMC mRNA in either group of rats, but this could be due to surprisingly low levels in the 6 mo AL group. Poly A RNA levels did not differ among treatment or age groups. These results are consistent with the hypothesis that the hypoadrenocorticism of the FR rat has physiological effects. They also indicate that the relatively high levels of POMC mRNA seen in AL rats are not essential for the extended lifespan of food restricted rats. (supported by a grant from the NIA).


A decrease in cortical 5-HT2 receptors is found in both aged humans and rats, but little is known of the functional properties of these receptors. In immature rats, NMDA induced depolarization of cortical neurons is enhanced by co-activation of 5-HT2 receptors (Rahman and Neuman, submitted). We now report this enhancement is dramatically reduced in 25 to 29 month old Fisher rats.

Wedges for "grease" gap recording were prepared from 500 µm thick slices of rat cortex (motor area). In young rats, amplitude of the NMDA (50 µM) depolarization was increased by co-administration of 5-HT (100 and 230% by 10 and 30 µM 5-HT respectively). In old rats 5-HT either had no effect (10 µM) or reduced the NMDA depolarization by 33% (30 µM). DOI (5 µM), a mixed 5-HT1C and 5-HT2 agonist, enhanced the NMDA response in young rats (175%) but not in old. Phenylephrine (10 µM) and carbobol (10 µM) enhanced the NMDA depolarization in both young (60 and 80% respectively) and old rats (82 and 162%). Functional 5-HT2 receptors are present in the cortex of old rats since bath application of atropine (0.1M, 40 min), a protein kinase C inhibitor, led to a 260% facilitation of the NMDA response by 30 µM 5-HT.

In conclusion, comparison of 5-HT2 with α1 and muscarinic effector systems demonstrates that the former is selectively reduced in old rats. We are currently using in situ hybridization to correlate 5-HT2 receptor message with functional changes.

Supported by the Medical Research Council (Canada)

TRANSFORMING GROWTH FACTOR-β1 mRNA INCREASES IN RAT AND HUMAN BRAIN WITH AGING. N.R. Nichoile, S.A. Johnson and C.E. Finch, Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles CA 90089-0191.

Transforming growth factor-β1 (TGF-β1) is a cytokine with well-known roles in differentiation and tissue repair. Previously, we cloned TGF-β1 from hippocampus of 3 mo old rats (Mol. Cell. Neurosci. 2:221, 1991). In the deafferented hypothalamus and striatum after lesioning (J. Neurosci. Res. 28:134, 1991). Both temporal and neauronatal expression suggested a role for TGF-β1 in synaptic plasticity and tissue repair in adult brain. Since infusion of TGF-β1 into the lateral ventricle resulted in an increase in GFAP mRNA and protein, we compared changes in hippocampal TGF-β1 mRNA during aging in FSBQ male rats with GFAP mRNA (positive control) by RNA blot hybridization analysis. In a single cohort of FSBQ male rats, TGF-β1 mRNA increased 50% in the hippocampus of 24 mo compared with 6 and 15 mo old rats. A similar increase at 24 mo was seen in both hippocampus and striatum of a second cohort of FSBQ male rats. Furthermore, hippocampal GFAP and TGF-β1 mRNA prevalence in individual rats are positively correlated when all age groups are considered together (r² = 0.634, P < 0.0001, n = 22). In young brain, GFAP and TGF-β1 mRNA prevalence also increase in total RNA samples from different cortical areas and hippocampus of old (73 ± 7) compared with young (41 ± 12) individuals. As in the rat, human GFAP and TGF-β1 mRNA are positively correlated (r² = 0.395, P = 0.0001, n = 52). Since an increase in TGF-β1 mRNA may result in a concomitant increase in bioactive peptide, these data suggest a role for this cytokine in glialosis and increased astrocytic reactivity contributing to age-related changes in synaptic plasticity. (Supported by NIH grant AG-07909)
AGING III

FRIDAY AM

625.3

DIMinished translational regulation of somatostatin mRNA contributes to the reduction in growth hormone secretion with age in Lymnaea stagnalis*. E. D’Costa, J.E. Lenham, and E. Ingram. Department of Physiology and Pharmacology, Bowmar Gray School of Medicine, University of Winston-Salem, Winston-Salem, NC 27157.

With advancing age, there is a decline in the capacity of tissues to synthesize and secrete growth hormone. This decline in protein synthetic capacity appears to be closely related to a decline in insulin-like growth factor-1 and growth hormone since these hormones are potent anabolic agents, decrease with age and administration of the hormones increase protein synthetic capacity in tissues. Previous studies have indicated that part of the diminution in the decline in the amplitude of growth hormone pulses is the result of an increase in somatostatin release from hypothalamic neurons. However, total somatostatin mRNA in the hypothalamus decreases substantially with age. In the present study, we compared polysomal bound and total somatostatin mRNA levels in Brown-Norway rats at 3 ages (6, 15 and 26 months) and in both ad libitum fed and dietary restricted animals. Total somatostatin mRNA decreased with age (p < 0.01) and this decline was prevented by dietary restriction. Polysomal somatostatin mRNA increased approximately 46% with age (p < 0.05). When data were expressed as polysomal bound somatostatin mRNA/total somatostatin mRNA, a substantial increase was observed in ad libitum fed animals (p < 0.01) which was not observed in dietary restricted animals. These results indicate that with age there is increased recruitment of somatostatin mRNA onto polysomes suggesting a loss of translational control.

Supported by NIH grant AG07752.

625.4

Different age-related decreases of ankyrin and spectrin occur in the mouse telencephalon. N. Lam, R.A. Bahr, A.C. Godshall, R. Granger*, and G. Lynch. Center for the Neurobiol. of Learning & Memory, Univ. of Calif., Irvine, CA 92717.

Deteriorating neuropathic processes are commonly associated with a decrease in neural activity and, eventually, brain function. It has been suggested that such neurodegenerative conditions may occur as a consequence of normal aging. The stable breakdown products of the membrane cytoskeletal protein spectrin, which act as biochemical markers correlating with the onset of many pathologic processes, have recently been shown to accumulate in the aged mouse telencephalon (Bahr et al., Neurosci. Lett. 133, 237, 1991). In accord with this study, antibodies to cytoskeletal constituents were used to screen telencephalic immunoblots from three (n = 13) and twenty-five (n = 13-15) month old mice. The immunobalancing of neuronal-200, talin, and Band 3, as measured by densitometric scanning, did not exhibit significant age-related changes, while actin decreased in the aged group as compared to the younger animals by a marginal 9.4 ± 4.5% (mean ± SEM, p < 0.06, two-tailed t-test). On the other hand, labelling of the α and β spectrin subunits decreased significantly with aging by 17.3 ± 3.5% (p < 0.01) and 18 ± 7.8% (p < 0.05), respectively. Labelling of the 220 kDa ankyrin had an age-related decrease of 37.5 ± 6.6% (p < 0.001) which is 2.1-fold greater than the decrease in α (p < 0.01) and β (p = 0.05) spectrin. In another group of mice, the differences in age effects on ankyrin and a spectrin was apparent between 3 (n = 4-5) and 10 (n = 4) months where the immuneactivity levels decreased 26% and 3%, respectively (p < 0.01). Between 10 and 20 (n = 4) months of age, both antigens decreased 8% while between 20 and 25 (n = 4) months ankyrin and a spectrin diminished 18% and 14%, respectively (p < 0.05), in conclusion, aging processes appear to act differently on ankyrin and spectrin as far as rate and magnitude of decline (Supported by grant NIA AG0058).

625.5

Effect of acetyl-L-carnitine on trauma-induced neuropathies in the young and aged rat: morphological, morphometric, functional evaluation. C. De Angelis, E. Frasca, P. Perini, G. Gis, M.T. Ramaccio*, L. Angelucci1. Institute for Research on Senescence, Sigma Tau, Pomezia, Rome; 1Institute of Pharmacology II, La Sapienza Univ. of Rome, Italy.

The favorable effects of acetyl-L-carnitine (ALCAR) on behavior and neuromorphological parameters of the CNS in aged rats prompted us to investigate its action on structure and function of intact and lesioned sciatic nerves in young and aged male Sprague Dawley rats. Sciatic nerve sections from animals sacrificed in anesthesia were stained with toluidine blue. In 24-month-old rats treated with ALCAR (150mg/kg/dy in drinking water) for 6 months, sciatic nerves contained a lower number of altered myelinated fibers (47%) and a higher number of normal fibers (+23%) than in age-matched controls. In rats induced injury caused 5 days later complete degeneration of the myelinated fibers. In both young and aged rats, treatment with ALCAR (15-60 days and 6-9 months, respectively) revealed an increased density of regenerating axons at 15 days (young) and 30 days (aged) after crush and an increased axonal size at 60 days. Also, the size of the entire myelinated fiber (diameter + axon) was larger in treated aged rats than in controls at 100 days after crush.

In agreement with the morphological features, the functional aspect (walking tracks in ALCAR-treated rats showed a faster recovery of deambulation. Thus, the neurotrophic action of ALCAR in the CNS is also exerted in the PNs of both young and aged rats, even in the presence of other etiopathogenetic factors, e.g. trauma.

625.6

Rate of change in size of abdominal ganglia as a function of weight in Aplysia C. J. Fries*, C. Hong, M. Stanley, J. Estes, S. West, R. Holt. George Mason University, Fairfax, VA 22003.

The relationship between abdominal ganglion size and weight was examined for 194 Aplysia C. whose weight ranged from 0.3 to 300 gms, by measuring the area of the abdominal ganglion. The area was determined using a compound microscope with calibrated objective. The best fit for the data required three different equations for the three weight ranges - 0.3 - 2.5, 2.5 - 7.5 and 7.5 - 300 gms. The equations were:

\[ y = \begin{cases} 0.3x + 0.25 \text{ gms} & \text{for } 0.3 \leq x \leq 2.5 \\ 2.5x + 7.5 \text{ gms} & \text{for } 2.5 < x \leq 7.5 \\ 7.5x + 300 \text{ gms} & \text{for } x > 7.5 \end{cases} \]

with a correlation coefficient of 0.97. Further analysis using dummy variables and curvilinear transforms will be performed. The transition points may be useful markers in development. Further analysis using dummy variables and curvilinear transforms will be performed. The transition points may be useful markers in development.}

625.7

Synaptic connections between identified neurons during aging of Aplysia C. Jane, W. C. Wilkerson and M. van der Roesth. SPOKE Foundation (Scientific Organizing Program for the Organization of Neuroscience), Department of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

Although many studies are known on age-related changes in the CNS, hardly any reports are available on the fate of synaptic connections between identified individual neurons during the animal’s life span. The pond snail Aplysia has a CNS with identifiable giant nerve cells permitting a life span approach to physiological studies of identified interneuronal synaptic contacts. This neurophysiological study reports on age-related changes in an electrical synapse between two giant peptidergic neurons (VDI and RPD2) and in chemical synaptic contacts of an identified dopamine containing giant neuron (RPD1). Experiments on VDI and RPD2 showed that effectiveness of the electrical synapses between these neurons decreases with age. In addition pacemaker properties changed. The actions of the different changes together can explain the irregularities in the firing characteristics of the VDI/RPD2 system as found in old animals. In the study of properties of chemical synaptic connections attention was focussed on one particular group of follower neurons of RPD1. These A-group neurons are situated in the right parietal ganglion. In young animals only a few A-neurons were found to receive synaptic input from RPD1 whereas in animals of 6 - 10 months of age A-neurons receiving synaptic input were commonly found. In old animals (older than 12 months) the number of A-neurons with synaptic input from RPD1 decreased. In Aplysia, electrical and chemical connectivity between neurons undergo a continuous change with age. Future experiments will be directed to the study of mechanisms governing these age-related changes.

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625.8


In previous studies we have examined age-related changes in astrocyte gene expression in the mouse brain and have reported a 40-80% increase in the message level for glial fibrillary acidic protein (GFAP) throughout the brain. To more fully understand the dynamics associated with this change, we employed a model of synaptic loss within the hippocampus by unilaterally transecting the fimbria-fornix pathway in 7 month old C57Bl/6 mice. Success of the lesions was evidenced by loss of acetycholinesterase staining in the hippocampus on the side of the lesion. In situ hybridization signals for GFAP RNA increased in both contralateral and ipsilateral hippocampi by 1 day post-lesion, peaked between 2 and 4 days post-lesion, was attenuated by 8 days post-lesion, and returned to non-lesioned control levels by 16 days post-lesion. Studies in progress are investigating this same lesion model in 3, 12, and 23 month old mice at 2, 5 and 10 days post-lesion. We will examine RNA for GFAP, glutamine synthase, apolipoprotein E, sulfated glycoprotein 2, 5-100, and alpha-1-antichymotrypsin. We have shown that in 7-month old mice have shown to have increased levels of RNA in astrocytes in various lesion models and/or neurodegenerative diseases. Supported by AG-07892, AG-00093, AHA-GIA 891079, and AHA-EIA 890173.
625.9

Increasing evidence suggests that microglia express immune-related antigens, function as antigen presenting cells and produce cytokines. Many aspects of peripheral immune function are reduced during aging, but little is known about microglia of the aged brain. Vibratome sections from F344/Brown-Norway F1 hybrid rats at 9, 18 or 24 mo of age were stained by lectin labeling with Ricinus Communis agglutinin I (RCA) or by immunocytochemistry using a monoclonal antibody against the rat MHC class II antigen (OX-6). The number of OX-6+ cellular profiles was counted and the percent area of tissue sections occupied by RCA reaction product was quantified using a video- and computer-based image analysis system.

The number of microglia expressing MHC class II antigen increased with aging in both gray and white matter. In neocortex, OX-6+ cells increased from 14±3 cells/hemisphere/section (mean±SEM, n=4) at 9 mo to 43±4 at 18 mo and 96±25 at 24 mo. The number of microglia expressing OX-6 also increased 3-fold in the hippocampal hilus (regio of analysis: 11±3; 18 mo: 28±4; 24 mo: 46±6 cells/0.25 mm²); and 5-fold in the corpus callosum (9 mo: 24±5; 18 mo: 57±8; 24 mo: 152±16 cells/0.25 mm²). OX-6+ cells also appeared larger in aged brain. RCA labeled a larger fraction of the microglial population than did OX-6. However, the percent area occupied by RCA reaction product was not increased as a function of age. These findings suggest some microglial markers remain stable during aging, but a larger fraction of microglia appear in an "activated" state, as more cells express MHC class II antigen. Supported by AG07892 & AHA-EIA 890173.

625.10
NEUROFILAMENT GENE EXPRESSION DECLINES IN THE DORSAL ROOT GANGLIA OF AGING RATS. L. Parish*, J.N. Scott, C.A. Krekulous, A.W. Clark, Departments of Pathology & Clinical Neurosciences, Univ. of Calgary, Calgary, Alberta, Canada.

Neurofilaments (NFs) are major structural components of neuronal cytoskeletal fibers. NF gene expression increases during maturation and is associated with an increase in NF content and caliber of axons. In this study we asked whether a decline in NF subunit gene expression occurs with aging, and whether this is correlated with axonal shrinkage. F344 rats were used at 4 ages (3, 5, 12, and 24 months; n=3 rats / age group). L4-L6 dorsal root ganglia (DRG) were processed for quantitative Northern and in situ hybridization with NF-L (light subunit) cDNA. Morphometric analysis was done of the L4 DRG and proximal dorsal root. Our results showed a 50% decrease in NF-L by Northern analysis at 24 months as compared to 3-12 months (Mann Whitney U, p < 0.02), and a similar decrease in NF-L mRNA grain density by in situ hybridization (n=100 neurons / age group, p < 0.001, t-test). No changes were seen in GAP-43 mRNA in these same samples with aging, by Northern or in situ hybridizations. Morphometric data showed a < 20% decrease in neuronal density in the DRG (p < 0.05), but no neuronal shrinkage (n=100 neurons / age group, p > 0.5, F-test). There was a 20% decrease in the diameter of large axons in the proximal dorsal roots at 24 months (n = 300 axons / age group; p < 0.01). These results indicate that NF mRNA declines with age and is associated with axonal shrinkage. Constitutive decline in NF gene expression can result in axonal shrinkage and may be a substrate for age associated neural degeneration.

625.11

It has recently been suggested that impairment of energy metabolism plays a role in neurodegenerative disorders. In this study, we tested the possibility that age-related changes in mitochondrial activity may predispose to neurodegeneration. Squirrel monkeys were divided into three groups according to their age: young (n=4; age 3 year old), middle age (n=5: age 10 year old), and old age (n=6 year old). Animals were sacrificed by CO2 asphyxiation and brains were removed and dissected. Mitochondria were immediately prepared from the caudate, putamen and cerebellar cortex. Mitochondrial activity was measured as formation of ATP in the presence of different substrates, namely pyruvate and malate or succinate with rotenone. A significant decrease in the rate of ATP synthesis was measured between young and middle age monkeys in all areas of the brain when pyruvate and malate were used as metabolic substrates. In contrast, in the presence of succinate and rotenone, the decline in mitochondrial activity was only evident after 12 hours. The decreased rate of ATP synthesis could be accounted for by a decrease in mitochondrial proteins in the tissues; indeed, the activity of other mitochondrial enzymes (i.e. citrate synthase and monomine oxidase A) remained unchanged in all age groups. Thus, impairment of energy metabolism occurs with aging and may be involved in neurodegenerative disorders of the elderly. Our data also suggest that mitochondrial Complex I activity may decline at a relatively earlier age than the activities of other enzyme complexes of the respiratory chain.

625.12

In brains of C57BL/6 (B6) mice, age-associated inclusions were described (M Jucker et al, Science, 255:1443, 1992) that resemble lesions reported in transgenic mice produced with a construct of human amyloid precursor protein 9 (Fragment (D Wirak et al, Science, 253:323, 1991). Inclusions in B6 mice were first identified immunohistochemically with an antibody to a 110 KD lamin B binding protein (LPB) that is also present in somata of neurons and dendrites. The antibody was specifically reactive with LPB that clusters in young mice (<6 mo) might provide clues to their identification in inclusions in astrocytic processes and in proximity to capillaries. Morphological analysis using combined LBP and GFAP staining to identify inclusions in astrocytic processes and in proximity to capillaries. Metaanalysis of the inclusions remains unidentifiable, but detection of tangle stained clusters in young mice (<6 mo) might provide clues to their identification. Preliminary behavioral and morphological analysis has found little relationship between inclusion formation and the density of hippocampal inclusions and glia cells in aged mice.

625.13

A novel peptide (HCNP: Ac-Val-Asn-Pro-Glu-Pro-Val-Ala) from rat hippocampus is involved in axonal growth and is a substrate for age associated neural degeneration. We have measured in autopsied brain of neurologically normal subjects, basal SAMDC activity, sensitivity to substrate, activator and inhibitor; regional distribution and influence of aging. In a preliminary experimental animal study, SAMDC activity in brain of transgenic mice cross hybrids (C 57BL/6: Age 10 months) was measured. Although S-ADENOSYLMETHIONINE DECARBOXYLASE IN HUMAN BRAIN. Lesley D. Lewis, D.M. Morris*, and Stephen J. Kish. Clarke Institute of Psychiatry, Toronto, Canada, MST 1HA.

S-adenosylmethionine decarboxylase (SAMDC) is a key regulatory enzyme in the biosynthesis of polyamines, substances which have been implicated as modulators of brain excitability. Little baseline information is available regarding SAMDC activity declined by 47% after 24 hours postmortem. The specific enzyme activity was measured in human brain was measured using a CO2 trapping procedure with a specific inhibitor of SAMDC activity (MDL 73811; IC50=31.5 mg/L) for blocks. The enzyme was characterized in parietal cortex with regard to substrate affinity (Km=53 µM), Vmax=74 pmol/CO2/h/mgP, and enzyme activity was markedly stimulated (+600%) by putrescine (Km=16.7 µM).

The distribution of SAMDC activity in 12 human brain areas was measured (n=5 brains/area, except white matter n=3). The highest activity was observed in occipital, parietal, frontal and temporal cortices (58.3, 48.1, 44.7 and 36.1 pmol/h/mgP), and enzyme activity was markedly stimulated (+600%) by putrescine (Km=16.7 µM).

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AGING III

625.15


Alzheimer's disease (AD) classically affects the hippocampus and association cortices. However, there has been some speculation that these areas are not first and, thus, most severely affected areas in a disease which proceeds progressively through the brain. Cerebellum, which is generally considered to be less affected, may exhibit early signs if the disease is progressive. RP-HPLC analysis of cerebellum from AD and control cases (73-86 years old) demonstrates elevated levels of fragments of β and β2 hemoglobin. In the AD cases, a percent change ranging from 59% to 164%. Immunocytochemical analysis of sections of cerebellum from most of the same cases shows human anti-hemoglobin immunoreactivity of granule cells and blood vessels. The granule cell staining appears to be membranous. The pattern of staining is similar in the AD and control cases although the staining is generally less intense in the control cases. One young control case (36 years old) shows blood vessel but no granule cell staining. This indicates that the localization of hemoglobin to the brain parenchyma may be an age-related phenomenon which is exaggerated in AD. The presence of hemoglobin in the brain parenchyma suggests the possibility of blood brain barrier malfunction in aging and AD cerebellum. Supported by R35AG01616 (R.S.).

625.16

DISTRIBUTION OF PROTEIN SP40-40-LIKE IMMUNOREACTIVITY IN THE RAT BRAIN: A ROLE AGAINST M. Senten, N.H. Chen (1), E. Janat, and Y. Lamoureux. INSERM U161, 75014 Paris, France; and (1) Showa University, Tokyo, Japan.

The protein SP40-40 is the human counterpart of the rat sulfated glycoprotein 2 (SP2), whose mRNA has a widespread expression in the developing and the mature rat brain. In the present study, the distribution pattern and the cellular localization of the SP40-40 protein were studied in various brain and spinal cord regions in young adult (3-4 months) Sprague-Dawley rats, using a well characterized polyclonal antibody. SP40-40-like immunoreactivity was mainly observed in the ganglia, the ependymal cells; a consistent immunolabelling was also observed in the cingulate cortex (mainly layer VI), the retrosplenial cortex (layers II-VI) as well as in the hypothalamus. The SP40-40-like immunoreactivity was mainly observed within cell somata and their processes as a diffuse reaction product. Double-labeling experiments performed with antiserum against GFAP demonstrated that SP40-40-like immunoreactivity was mainly localized within neurons. Although the function of protein SP40-40 in the central nervous system still remains to be elucidated, its involvement in neuronal death has been suggested. Therefore, we sought to determine the possible implication of SP40-40 in age-related cell death and apoptosis. We performed a similar anatomical study in 12, 20-22 and 30-31 months old rats. With increasing age SP40-40-like immunoreactivity increased or appeared in specific brain areas (cerebral cortex, thalamus, hypothalamus, red nucleus, superior colliculus, olivary nucleus, cerebellum, cranial nerve nuclei). However, these changes did not seem to be associated with obvious signs of neuronal death or degeneration.

NEUROGLIA AND MYELIN V

626.1

[Ca2+]-TRANSIENTS IN CULTURED SCHWANN CELLS EVOKED BY ACTIVATION OF NICOTINIC AChR's. E. Yoder*, V. Lev-Ram†, and M. H. Ellisman. San Diego Microscopy and Imaging Resource, Departments of Neurosciences and Pharmacology, University of California, San Diego, La Jolla, CA, 92035-0008.

[Ca2+]i transients are known to mediate many cellular processes in developing and mature glia of the CNS. In the PNS, transient increases in [Ca2+]i have been reported to occur in myelinating and terminal Schwann cells following axonal activation (Pei et al., J. Neurochem. 62, 1591, 1991; Aumers et al., Neurosci. Lett. 90, 90, 1991); the physiological role of these transients is unknown. In order to gain insight into the role of these transients, we have examined [Ca2+]i transients in primary cultures of Schwann cells from neonatal rat sciatic nerves. Experiments were performed using cells loaded with the calcium indicator dye fluo-3 introduced via an AM ester, or a calcium indicator dye that bound to the plasma membrane. These experiments found to induce a transient increase in [Ca2+]i in many cells. Some exhibited an oscillation in [Ca2+]i, in response to carbamol. These effects were mimicked by nicotine and blocked by tubocurarine, but were not blocked by atropine. Thus, the observed action of ACh is via nicotinic (nAChR) rather than muscarinic AC receptors. Nicotine induced [Ca2+]i transients were observed in the absence of [Ca2+]i plus BTA indicating that the Ca2+ transient results from the release of Ca2+ from intracellular stores. The nature of this Ca2+ store was investigated by pharmacological manipulation. Thapsigargin stimulated an increase in [Ca2+]i whereas caffeine did not. These results suggest that the release of Ca2+ from an intracellular store in cultured Schwann cells may be mediated by inositol trisphosphate receptors rather than ryanodine receptors. Mechanisms linking release of Ca2+ from intracellular stores in response to nAChR stimulation in Schwann cells but as well the functional significance of such stimulation remain to be determined.

626.3

ASTROCYTES INHIBIT SCHWANN CELL PROLIFERATION AND MYE LINATION OF DORSAL ROOT GANGLION NEURONS IN VITRO. V. Gschwend, P. Hong, and R. Hurn. The Miami Project to Cure Paralysis & Department of Neurosurgery, University of Miami School of Medicine, Miami, FL.

Schwann cells (SCs) aid central nervous system regeneration. However, little is known about the effect CNS glia may have on SC function. In the present study, the effect of astrocytes (AS) on SC proliferation and myelination was evaluated using pure neuronal cultures [prepared from diencephalic encephalophic rat dorsal root ganglia (DRG)] which were seeded with pure SC (50 x 10^3 cells/culture) isolated from neonatal rat sciatic nerve. SC function in these DRG-SC cocultures was assessed 48 hours after seeding. AS or Fbs (0, 150 or 500 x 10^3 cells) were added to DRG-SC cultures; the cultures were maintained in medium containing 1% FBS. Soluble factors released by AS induced a 27% decrease in SC expression of GalC was blocked, but that of O4 was not. To determine if AS-SC interactions are linked to calcium regulation and, if so, to examine the temporal relationship between the expression of receptors for various ligands on Schwann cell differentiation.

Schwann cell cultures were prepared from neonatal rat sciatic nerve and after 6 hours, 1d, 2d, 4d, and 14 days in vitro (DIV) were loaded with the calcium indicator dye, fura 2-AM. The influence of up to eight different neurophins on Ca2+ levels was examined using a video-based imaging system. Neurophins that elevated Ca2+ levels included those that are present in the adult sciatic nerve. In contrast, neurophins that elevated Ca2+ levels were bradykinin (BK; 10^-7M) and adenosine triphosphate (ATP; 10^-M). Both ATP and BK increased Schwann cell calcium levels within 15 seconds of drug addition, after which Ca2+ levels decreased toward baseline over 1-2 minutes. Calcium levels also increased in Schwann cells exposed to histamine (10^{-M}) and glutamate (10^{-M}). However, a smaller and more variable percentage of mature Schwann cell responses to various ligands on Schwann cell differentiation. Schwann cells responded to these ligands. The results of these studies indicate that subpopulations of S100B immunopositive Schwann cells respond to one or more neurophins with a rise in Ca2+ levels. Furthermore, it appears that developmental processes occurring in vivo influence the percentage of Schwann cells responding to different neurophins with a rise in Ca2+ levels.

626.4

IN VIVO IDENTIFICATION OF SCHWANN CELL NUCLEI IN MOUSE NEUROMUSCULAR JUNCTIONS. S. Nakashiro*, and N. Robbins. Dept. of Neurosciences, Case Western Reserve Univ., Cleveland, OH 44118.

Little is known about the function of the terminal Schwann cell at the neuromuscular junction (NMJ). One strategy is to assess the function of this cell before and after depletion in vivo. For this reason, we have developed a technique to identify terminal Schwann cell nuclei in living NMJs. Superfine electrodes in the mouse pectineus muscle were identified by staining the synaptic matrix with fluorescein-conjugated dianino vicina agglutinin, and nuclei were stained with Hoechst 33342 in the living animal. By shape and location of the nuclei at the NMJ and the features of their nuclei, the Schwann cell nuclei were then distinguishable from those of other cells such as muscle, fibroblasts, and capillary endothelium. This was confirmed through EM studies of serial sections of NMJ's. The Schwann cell nuclei, for example, have several small nucleoli which are also seen in Hoescht-stained nuclei above the nerve terminal, whereas muscle nuclei near NMJ's have only one or two prominent nucleoli. This identification was confirmed by S-100 immunostaining for Schwann cells in whole mounts. With this technique, we are now able to destroy the nucleus of the terminal Schwann cell in vivo using a focused laser beam. Deletion techniques such as these may provide access to the terminal Schwann cell in the maintenance and plasticity of the neuromuscular junction. Supported by NIA grants AG08886 and AG06641.
626.5 UPTAKE OF AXONALLY TRANSPORTED LATEX NANOSPHERES BY PARANODAL AXON-SCHWANN CELL NETWORKS. K.P. Gutowska* and H. Persson. Dept. of Anatomy, Univ. of Göteborg, 413 90 Göteborg, Sweden.

The distribution of retrogradely transported red-fluorescent latex nanospheres (Molecular Probes) was studied by light and electron microscopy in lumbosacral ventral root axons of adult rats. The left sciatic nerve was crushed and immediately injected intramuscularly with 50 μl of the tracer. After a 48-72 h postinjection survival period, the animals were perfused fixed with 4% paraformaldehyde. Vibratome- to cut ventral root sections were examined with a Nikon FXA epifluorescence microscope. Spheroid granules exhibiting red fluorescence were distributed within many axons of the injected but not the control side. The granules were often concentrated at nodes of Ranvier, where they were situated in close association with the paranodal myelin sheath. For electron microscopic detection of elements showing this type of fluorescence, photoconversion was performed in epifluorescent light using a solution of 0.1% DAB in Tris buffer. With this procedure a black precipitate, which appeared electron dense at the ultrastructural level, was formed in association with membrane-delimited organelles of various sizes. In the intermodal parts of the axons organelles of this appearance were situated in the axoplasm. By contrast, most organelles in association with the paranodal myelin sheath were situated within the so-called axon-Schwann cell network, thereby being segregated from the main axoplasm.

Our results show that axonally transported non-neuronal materials can be removed from motor axons via a transport pathway within paranodal axon-Schwann cell networks. Most likely, this process represents a local mechanism whereby motor neurons can eliminate retrogradely transported foreign substances before arriving to the neuronal perikaryon in the CNS.

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626.7 DETECTION AND REGULATION OF PMP-22 mRNA AND PROTEIN IN CULTURED SCHWANN CELLS. S. Persip, U. Suter, G. J. Snipes*, A. A. Welch*. E. E. B. Dept. of Anatomy, and Cell Biology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.* Dept. of Neurobiology, Stanford Univ. School of Medicine, Stanford, CA 94305-5401, USA.

Peripheral myelin protein-22kDa (PMP-22) is a novel myelin-membrane associated glycoprotein that has been localized within Schwann cells of sciatic nerve (Snipes et al., J. Cell Biol., 117, 225, 1992). The function of PMP-22 is unknown, however recent studies suggest that a point mutation in PMP-22 might cause congenital stationary night blindness (Suter et al., Nature, 355, 241, 1992). In this study, we have analyzed PMP-22 in cultured rat sciatic nerve Schwann cells to determine whether these cells express the protein in the absence of neurons and whether the controls that regulate its production are similar to those that regulate production of other myelin-associated proteins. Immunocytochemical studies using polyclonal antibodies raised against synthetic peptides of sequences within the protein show that PMP-22-like immunoreactivity is present within Schwann cells. Northern blot and RNase protection assays reveal that mRNA coding for PMP-22 is present in Schwann cells cultured in serum-containing medium supplemented with glial growth factor and forskolin. In the absence of glucocorticoids, mRNA levels decline but are re-established within 36 hours after forskolin is added to the medium. Increases in PMP-22 protein levels also occurred following forskolin treatment as determined in studies in which Schwann cells were metabolically labelled with [35S-methionine and cellular extracts were immunoprecipitated with anti-PMP-22 antibodies. Taken together, our results suggest that PMP-22 is present in axon-free Schwann cells and its expression is regulated in a manner similar to that of other myelin-associated proteins such as MBP and P0.

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SCIP (also known as tist-1 and Oct-6) is a POU transcription factor that is expressed by Schwann cells and appears to down-regulate the expression of myelin genes. We have analyzed the expression of SCP mRNA in the peripheral nervous system of the rat. In developing nerves, the steady state level of SCIP mRNA was highest at the day of birth, and fell quickly to a level that persisted into adulthood. We examined the steady state level of SCIP mRNA in adult sciatic nerves that were either permanently transected (to cause Wallerian degeneration axonal regeneration) or crushed (to cause Wallerian degeneration but allow axonal regeneration). After nerve-transection, SCIP mRNA increased slightly and transiently. A steady state level of SCIP was maintained for at least 58 days. Since Wallerian degeneration in crushed and transected nerves appears to be similar, the difference in SCIP expression appears to be related to regenerating axons. Thus, we believe that regenerating axons in crushed nerves interact with Schwann cells, leading to up-regulation of SCIP mRNA.

626.9 THE EFFECTS OF ETHANOL ON TRANSFECTED SCHWANN CELLS. B.M. Labiassiere, S. Moore, A. Springler, P.W. Armstrong and W. Knight. The University of Virginia State Univ. Petersburg, VA. 23806

Primary Schwann Cells transfected with the SV-40 T-antigen provides a model for obtaining cells that express properties associated with normal Schwann cells in culture. Ethanol has been shown to effect the nervous system, leading to alcohol-related peripheral neuropathy. The effects of various concentrations of ethanol on the morphology, proliferation and protein synthesis of the transfected Schwann cell were investigated. Increases in ethanol treatments (22, 43, 86, and 122 mM) caused a decrease in cell proliferation and protein synthesis. Light microscopic evaluation revealed cells that were considered to be stained as the ethanol concentrations increased. These data indicate that transfected Schwann cells may provide an excellent model for studies of ethanol induced neuropathy in Schwann cells.

626.10 PHENOTYPIC FEATURES OF OLFACTORY ENSEATHING CELLS: AN IMMUNOCYTOCHEMICAL EXAMINATION. R. Duquette* and R. Dvorak. Dept. of Anatomy, Queen’s College of Medicine, and Department of Oral Biology, College of Dentistry, University of Saskatchewan, Saskatoon, Sask., Canada.

Olfactory ensheathing cells mix a variety of Schwann cell and astrocytic phenotypic features and are the only glia that ensheath the olfactory axons. The objective of this study was to determine what additional phenotypic features ensheathing cells would express when grown under in vitro conditions known to be optimal for the growth-differentiation of Schwann cells or astrocytes. Ensheathing cells were obtained from the nerve fiber layer of the olfactory bulb of E18 rat embryos. For the first experiment, ensheathing cells were grown in either Bottenstein’s G5 medium or in DMEM/F12/1%FBS/0.25 mM dBCAMP. Although dBCAMP did induce the appearance of weak GFAP-like immunoreactivity in these cells, they did not differentiate into typical astrocytes even when grown in either medium for several weeks. The second experiment examined whether ensheathing cells would assume a myelinating phenotype (like Schwann cells) after being plated onto purified DRG neuronal cultures. By four weeks many of the S100-positive ensheathing cells were Gal-C+ and MBP+ and had begun to myelinate the larger axons, as visualized with the electron microscope. These myelinating glia were not residual Schwann cells that had survived the antimitic treatment because control pure neuronal cultures contained no glial cells and no evidence of myelination. Thus, ensheathing cells can assume a myelinating phenotype in vitro. (This work was supported by a grant from the MRC of Canada).
626.11 GLIAL CELLS IN THE DEVELOPING OPTIC NERVE OF THE FROG LITORIA (HYLA) MOOREI, D.E. Playford* and S.A. Dunlop, Neurobiology Lab., Department of Psychology, University of Western Australia, Nedlands 6009.

We have recently shown that there is a biphasic sequence of myelination in the optic nerve of Litoria moorei. The first phase is initiated at the optic foramen in mid-tadpole life, spreads towards the eye and chiasm and is complete at metamorphic climax; the second phase is initiated at the chiasm, spreads towards the eye and results in approximately 2.5% of optic axons being myelinated in the fully mature adult. We have also reported that numbers of glial cells increase throughout life and that the proportion of oligodendrocytes mirrors the patterns of myelination (Playford & Dunlop, 1991, Neurosci. Abs. 17, 157,11). Here, we have examined glial cells ultrastructurally and show that although astrocytes and oligodendrocytes can be distinguished from mid-tadpole life, they continue to mature morphologically until the final adult stage. To determine when and where glial cell division is occurring, animals were injected with tritiated thymidine and killed 4-6 hours later. Dividing cells were seen at all levels in the nerve suggesting that differentiated astrocytes and oligodendrocytes undergo cell division. In addition to differentiated glial cells, there is a distinctive group of undifferentiated glial cells at the chiasmal end of the nerve. Serial reconstruction of wax sections shows that these undifferentiated cells eminate from the pre-optic recess, pass through the chiasm, enter the nerve as a dural cell mass and extend it for up to 50 microns. This cell mass diminishes after metamorphic climax. Dividing cells were also seen within the undifferentiated cell mass.

Funded by the National Health & Medical Research Council, Australia.

626.12 POTASSIUM CURRENTS FROM HEALTHY AND NEUROFIBROMA-DERIVED SCHWANN CELLS IN THE BICOLOR DAMSELFISH, A MODEL OF HUMAN NEUROFIBROMATOSIS. L.A. Fasler* and M.C. Schmit, Univ. of Miami Rosenstiel School of Marine and Atmospheric Science, NEIHS Marine and freshwater biomedical Science Center, Miami, Fl 33149.

Schwann cells are the predominant cell type observed in neurofibromas in Type 1 neurofibromatosis (NF-1) in man. The development of this disease is marked by changes in morphology and growth patterns in Schwann cells which may have physiological correlates. Here we present electrophysiological results on cultured Schwann cells from the only naturally occurring model of NF-1, damselfish neurofibromatosis (DNF), from the bicolor damselfish (Pomacentrus paruitus). Ionic currents were recorded using the whole cell patch clamp technique from cell bodies of healthy fish Schwann cells from peripheral nerve explant-derived cultures with or without axonal fragments present. These were compared to currents in cultured Schwann-like cells from neurofibromas. Each of these cell types expresses the glial specific antigen, S-100, but not fibronectin, an antigen found in both mammalian and damselfish perineurial cells. Multiple types of potassium currents were observed in both healthy and tumor derived cells, but with different currents predominating. All tumor derived cells had a transient current with a steep, sigmoid activation curve, which was partially blocked by external tetraethylammonium (TEA; 14 mM). This current is similar to A-type potassium current. Tumor derived cells often had only this current. All Schwann cells from healthy fish, regardless of the presence of an axonal fragment, had a delayed rectifier current, and often this current had little or no apparent inactivating component. This current was blocked by external TEA (14 mM). Supported by USPHS grants ES05705 and CA50313.


The olfactory organ of the spiny lobster, Panulirus argus, consists of a dense array of aesthetasc sensilla on the lateral filament of the antennule. Each sensillum contains the dendrites of several hundred chemosensory cells, and processes of a number of glial-like auxiliary cells. Electrophysiological studies have shown that sensilla include populations of receptor cells that respond to a variety of amino acids, including glycine, glutamate, and alanine. Biochemical studies have shown that sensilla also contain amino acid uptake systems as well as aminotransferases, and related enzymes. Messenger RNA isolated from the lobster's olfactory organ was used to construct a cDNA library in the vector ZAPFII. The library was screened for olfactory enzymes by means of a complementary chain reaction (PCR). Sequencing of two PCR products revealed an open reading frame of 1083 nucleotides, coding for 361 amino acids showing 63% identity to the enzyme glutamine synthetase (GS) from Drosophila melanogaster. Immunohistochemical and in situ hybridization studies showed that, in the brain, GS is localized in glia, and in the olfactory organ, GS is localized in the auxiliary cells. Biochemical studies showed that the olfactory sensilla have 4-fold greater GS activity than does lobster brain. Olfactory GS may play a role in controlling the background levels of amino acids in the environment of olfactory receptor cells. Supported by grants from the NSF (NSF BNS 8901337 & 8914602) and the Univ. of Fla. (D-50-8990).

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BLOOD-BRAIN BARRIER III

627.3

UPERGULATION OF THE BLOOD-BRAIN BARRIER (BBB) GLUCOSE TRANSPORTER (GLUT1) mRNA BY GLUCOSE DEPRIVATION. L. Wang, R.J. Baudo, W.H. Oldendorf*, and W.M. Partridge. Departments of Medicine and Neurology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The absence of neuroglucopenia symptoms in chronic hypoglycemia may be due to upregulation of the BBB GLUT1 glucose transporter. Therefore, we investigated the effect of glucose deprivation on the abundance of the GLUT1 transcript in bovine brain capillary endothelial cells in culture. The GLUT1 gene is over-expressed in cell culture. Paradoxically, there is a marked downregulation of GLUT1 gene expression in brain ECL (Baudo & Partridge, Mol. Cell. Neurosci., 1:224, 1990).

627.4


ET-1, synthesized predominantly by endothelial cells (ECs), is well-known to play a powerful role as a constrictor of vascular smooth muscle cells although less is known about possible ET-1 effects on other cell types. By analogy to another EC product, endothelin derived relaxing factor (NO), ET-1 could play a role as a neuromodulator in the nervous system. The objective of this study is to determine the possible role of ET-1 on the cerebral microcirculation characterizing ET-1 receptors on isolated microvessels (MV). The results of the radioisotope binding experiments showed that in MVs from the rabbit binding capacity is 827 fmol/mg and the Kd is 1.31/nM. In addition, ET-1 (10-1000 nM) significantly (p<0.05) stimulates Na+-dependent neutral amino acid transport as measured by the uptake of methyl amino isobutyric acid. Furthermore, we have shown that in aged rats (>18 mths) the number of ET-1 receptors decreases approximately 50% with no change in Kd. Our results demonstrate that the cerebral microcirculation i.e. the BBB, possesses receptors for ET-1 and that in response to this peptide, the BBB may be altered. In addition, in aged animals there is a decrease in the ET-1 receptor. These data suggest that BBB functions, such as amino acid transport, may change in age, which could enhance the toxicity of drugs administered.

627.5


Chronic exposure to nicotine has been linked to morphologic changes in aortic and umbilical cord endothelial cells. To study its effects in brain, we treated guinea pigs with nicotine for 14 days (Alzet minipumps; final plasma level: nicotine = 11.4 ng/ml; cotinine (nicotine metabolite) = 101.8 ng/ml) and examined endothelial cells either within (cerebral cortex) or outside (umbilical cord) the blood-brain barrier (BBB). Animals were perfused with aldehydes fixative and processed for electron microscopy. Quantiative comparisons of cortical endothelial cells from nicotine-treated (n=5) and control (n=3) animals revealed no obvious morphologic differences; capillaries from nicotine-treated neurohypophyses did, however, show a striking increase in microvillous-like projections into the capillary lumen. Quantiative ultrastructural morphometric analyses of neurohypophyseal capillaries determined that capillary endothelial cells displayed increased microvilli, increased endothelial vesicle number and the density of microvillus like luminal projections. We found a significant increase in microvillous-like luminal projections (η = 1.001). There were no consistent changes in the other parameters. The lack of morphologic alteration of BBB endothelial cells may reflect either subthreshold effects of nicotine or a different response of these cells to the toxin. Nicotine exposure alters both circulating vasopressin levels (Larose et al, J Pharm Exp Ther, 1988, 244:1093) and blood-brain vasopressin transport (Lipovac et al, Soc Neurosci Abstr, 1992). Thus, neurophysiologic changes may reflect either nicotine-induced alterations specifically related to vasopressinergic changes, or a generalized response of non-BBB endothelial cells to nicotine. (Tobacco Related Disease Research, CA, #ST0070).

627.6

MOPHOLOGICAL ALTERATIONS IN NEUROHYPOPHYSIAL ENDO­THELIAL CELLS OF NICOTINE-TREATED GUINEA PIGS. E. Barrón, M.N. Lipovac*, B.V. Zlokovic*, J.L. Johnson3* and L.S. Perlmutter1,2, Dept. of Neurosurgery, Univ. of California and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Chronic nicotine treatment may affect neuroendocrine function. We studied morphologic alterations in neurohypophyseal capillaries of guinea pigs treated with nicotine by capillary depletion method (control), i.e. by 117% as determined in capillary depleted brain tissue. A ten times greater rate of AVP capillary in situ sequestration was found as a result of AVP binding and transport vs. binding. The rate (Km) of [3H]-AVP entry into brains of N-treated animals was significantly increased in comparison to N-naive animals (control), i.e. by 2.4 ± 0.2 (mean ± SE, n = 3) fold increase in the GLUT1/actin mRNA ratio. This increase was time dependent and the maximum effect was observed at 20-24 hours after the hexose deprivation. Nuclear transcription run on assay showed no changes in neither the GLUT1 or actin gene transcription rate 24 hours after glucose deprivation.

Conclusion: i) glucose deprivation increases the abundance of GLUT1 mRNA in brain capillary endothelium, ii) this increase is probably due to enhanced stability of GLUT1 mRNA without changes in the gene transcriptional rate, and iii) this may represent the initial step in the upregulation of the BBB GLUT1 glucose transporter in chronic hypoglycemia.

627.7


Recent studies suggest that plasma proteins may enter the brain by vascular route via the CSF. In this study we immunolocalized IgG in neurons of the normal rat brain and examined the relationship of these labelled cells to the blood-brain and blood-CSF barriers. IgG was immunolocalized in brain sections using a biotinylated rabbit anti-IgG. Immunolabel was detected with the avidin-biotin-peroxidase complex and diaminobenzidine reaction. Immunostaining of these labelled cells was compared with the vascular wall and adjacent extracellular space. Neurons adjacent to these blood vessels were frequently immunolabelled. A limited number of blood vessels, scattered throughout the brain, exhibited immunoreactivity. The label was associated with the vascular wall and the adjacent extracellular space. Neurons adjacent to these blood vessels also were immunolabelled. IgG was also immunolocalized in neurons in the external granular layer of the parahippocampal gyrus, in the neocortex and in Purkinje cells in the cerebellum. These immunolabelled neurons were randomly distributed and were not typically bilateral in their distribution. The most unifying feature of these labelled neurons was that they were either intimately associated with an immunolabelled blood vessel or that their processes were in close proximity to a blood vessel. These findings suggest that i) the blood-brain barrier expresses limited permeability to IgG and 2) neurons may accumulate IgG from these permeable vascular sites and/or from the CSF.
BLOOD-BRAIN BARRIER III

627.9

This study was conducted to explore the effects of intra-ischemic mild hypothermia on blood-brain barrier (BBB) opening in a rodent model of MCA occlusion. Male SHR rats were subjected to permanent right common carotid artery occlusion and 2 hrs of reversible right MCA occlusion. Normothermic and hypothermic control groups were kept at 37.5°C throughout ischemia and reperfusion (RP). The hypothermic group was kept at 32°C under anesthesia during the 2 hrs of ischemia, but was kept at 37.5°C throughout RP. Following RP, at 2 min, 20 min or 46 hrs, a 1H-sucrose solution (20 µCi/100gm) was administered IV, circulated for 30 min, and the animals immediately sacrificed. Cortical transfer constants (Ki) for BBB permeation of sucrose were calculated from the ratio of parenchymal (dpm-g^-1) and time-integrated plasma (dpm-s-mL^-1) sucrose concentrations. A one-way ANOVA was used.

Ki Mean ± SE (n): *p<0.05,**p<0.01,***p<0.001.

RP:2 min RPs
Normothermia (8) 3.8 ± 0.5 6.7 ± 1.0 25.5 ± 3.4
Halothane (4) 2.6 ± 0.2* 7.0 ± 1.7
Hypothermia (6) 1.7 ± 0.2** 2.5 ± 0.6** 2.7 ± 0.7**

Intra-ischemic mild hypothermia protects against BBB breakdown, affording an explanation for hypothermic cytoprotection. Mild hypothermia should be employed to prevent vasogenic edema during thrombolytic therapy for stroke patients.

627.11
PROTEIN KINASE C (PKC) IN ISOLATED MICROVESSELS (MVs) IN AGING AND ALZHEIMER’S DISEASE (AD). F. Moore1, F. Grammas2, T. Buchtel1, A. Eroles1, M. Balf1, R. Leach1. Univeristy of Old's HSC, OR, 971021; Wayne St Univ, Detroit, MI 482012, Oregon HSC, Portland, OR 972013.

PKC, an important enzyme in signal transduction, is a primary factor in determining cellular responsiveness to receptor activation. Several studies have shown changes in enzyme activity, distribution or isoforms in the brain in aging and AD. To determine the PKC in the cerebral microcirculation from AD patients and controls and young and aged (>10 mths) rodents. MVs were isolated from the frontal, temporal, and parietal cortex of AD patients and age-matched controls (post-mortem time 6-12 hrs) and cytosolic and particulate fractions prepared. Evaluation of PKC activity after partial purification on Q-Sepharose indicates that PKC activity in AD MVs (β 64±2.9 pmol/mg/min) was significantly (p<0.05) less (34.7%) than that of controls MVs (16.1 1±- 6.1). In contrast, analysis of MVs from the cerebral cortex of young and aged rodents, by phosphol ester binding, indicates no significant difference in PKC. Our results demonstrate that PKC activity can be determined in human post-mortem samples and that AD microvessels demonstrate significantly less PKC activity compared to age-matched controls. In addition, PKC appears comparable in MVs from young vs. aged rodents. These data suggest that PKC function and responsiveness may be abnormal in AD. (Supported by AHA, OCAST, NIH NS 30457, and NIH P30AG08017).

627.12

PKC, a cytosolic enzyme important for receptor-mediated cell activation, localizes to the membrane upon activation. The distribution of PKC isoforms appears to be tissue and cell specific. The objective of this study was to determine PKC isoforms in the cerebral microvessels. MVs were isolated from the cerebral cortex of young and aged (>18 mths) rats as well as human autopsy specimens (parietal, temporal, frontal cortex) from Alzheimer’s AD) patients and controls. Cytosolic and particulate fractions were prepared, solubilized in SDS buffer and Western blots run using polyclonal antibodies to PKC isoforms α, β and γ. The results indicate that in both rat and human MVs, β is the most abundant and is present in both membrane and cytosolic fractions. The γ isoform is also present although to a much lower level than the β and α. This is consistent with studies in both rat and human MVs. Finally, the γ isoform was undetectable in rat and human MVs in either fraction. These data demonstrate that both α and β isoforms are present in the cerebral microcirculation and that β is the predominant species. Similarities in the distribution of isoforms in aging and young MVs as well as AD and control MVs suggest that altered PKC responsiveness in aging or AD may reflect changes in enzyme activity or level rather than a change in isoform type. (Supported by AHA, OCAST, NIH NS 30457 and VA Res).
ASSOCIATIVE CONDITIONING DOES NOT FACILITATE THE INDUCTION OF HETERO SYNAPTIC LONG-TERM DEPRESSION IN HIPPOCAMPAL FIELD CA1.

D.S. Kost* and W.C. Abraham. Department of Psychology and Neuroscience Research Center, University of Otago, Dunedin, New Zealand.

In vivo studies were conducted in order to assess differences in non-associative and associative heterosynaptic long-term depression (LTD) in region CA1. Recent reports have indicated that the presence of negatively correlated co-activity in CA1 inputs during stimulation of these neurons, converging CA1 inputs, LTD of uncorrelated synapses that is differentiable from non-associative LTD (Stanton and Szejnovics, Nature:339,1989; Stanton et al., Neurosci. Lett.:127,1991). Two-way stimulation protocols were employed in which one stratum radiatum input received pairwise stimulation consisting of trains of brief, high frequency bursts delivered 200 ms apart, sufficient to produce homosynaptic LTD, while another (separate) stratum radiatum input received either single pulses interleaved between straining bursts (associative condition) or no activity at all (non-associative condition). These basic procedures were conducted under a variety of conditions, including pre-conditioning ("priming") of the test pathway with 5 Hz stimulation, reduction of synaptic inhibition by perfusion with picrotoxin, and blockade of NMDA-receptor mediated responses with APV. LTD reversal studies were also carried out in order to assess associative conditioning procedures on non-neuronal pathways. Under no conditions were we able to induce associative LTD different from or greater than that non-associative LTD which has been generally observed in this region. However, the data do indicate that either mild, tonic or prior synaptically activity at the frequencies can affect subsequent synaptic weight changes. (Supported by New Zealand Health Research Council, Postdoctoral Fellowship grant to D.S.K.).
628.5

**SIMULTANEOUS EXPRESSION OF LTP BY AMPA AND NMDA RECEPTOR-MEDIATED EPSPS IS OF SIMILAR MAGNITUDE IN HIPPOCAMPAL PYRAMIDAL NEURONES.**

A.P. Southan, S.L. Olofsson, & D.G. Owen*. Wyeth Research (UK), Huntercombe Lane South, Taplow, Berks. SL6 0PH, UK.

Brief application of K+ channel blockers to CA1 neurones of the in vitro rat hippocampal slice has been reported to induce a long lasting (TEA, MCDP) or decremental (4-AP) potentiation of synaptic responses (Boyett & Ben-Ari, 1991; Nature 349, 67-69). In this study we have extended these observations to include dento-hippocampal (Dx) slices homogenised from the cortex of green and black marmoset, which are points and sex-specific blocks of voltage-activated K+ currents.

**Extractable field potential responses were recorded from the CA1 region of hippocampal slices using conventional methods and solutions. Potentials were evoked by constant stimulation of the Schaffer collaterals at 0.1 Hz/0.02ms duration, at 30°C. Drugs were applied in the perfusing medium for a period of ten minutes, slices would then be returned to the baseline condition.**

4-AP (10 mM), Toxin I (100 mM) and TEA (25mM) induced repetitive firing and increased the amplitude of the first population spike by 165%, 24% and 23% respectively, immediately following a ten minute exposure (n=5 in each case). The effect of TEA was rapid in onset but slowly reversed during washout. The enhancement due to TEA (peak ca 225% at 15 min wash) was slightly slower in onset and was poorly reversible (ca 150% enhancement at 60 min wash). Slices treated with Toxin I exhibited a slowly developing persistent potentiation which peaked 15 min after removal of the toxin (100% enhancement at 60 min wash). Although MCDP (1uM), γ-Dxs (60mM) and 8-Dxs (10mM) did not induce multiple population spikes (n=3 for each drug), MCDP promoted a rapid and persistent enhancement of the population spike amplitude (ca 200% at 60 min wash) and 8-Dxs produced a slowly developing enhancement taking up to 60 min to reach a maximum (ca 100% enhancement). γ-Dxs had similar actions as 8-Dxs but was much less potent (ca 40% enhancement at 60 min wash).

The qualitative differences in activity of the toxins may reflect relative selectivities for pre- and post-synaptic elements.

628.6

**DENDROTOKINS INDUCE A FORM OF LONG LASTING SYNAPTIC POTENTIATION IN HIPPOCAMPAL PYRAMIDAL NEURONES.**

A.P. Southan & D.G. Owen*. Wycherley Research (UK), Huntercombe Lane South, Taplow, Berks. SL6 0PH, UK.

**A novel technique for electrically evoking field responses in rat hippocampal slices (Ari, 1991; Nature 349, 67-69). In this study we have extended these observations to hippocampal slices of guinea-pig, cats, rats, mice, and rabbits.**

**Brief application of a K+ channel blocker to CA1 neurones of the in vitro rat hippocampal slice has been reported to induce a long lasting (TEA, MCDP) or decremental (4-AP) potentiation of synaptic responses (Boyett & Ben-Ari, 1991; Nature 349, 67-69). In this study we have extended these observations to include dento-hippocampal (Dx)游泳tell homogenised from the cortex of green and black marmoset, which are points and sex-specific blocks of voltage-activated K+ currents.**

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The qualitative differences in activity of the toxins may reflect relative selectivities for pre- and post-synaptic elements.

628.7

**ETHANOL-INDUCED SUPPRESSION OF LONG-TERM POTENTIATION IN THE DENTATE GYRUS IS REVERSED BY LESIONS TO THE SEPTOTRIGONAL NUCLEUS.**


**We have previously reported that phorbol esters (PE) cause an increase in the frequency of spontaneous miniature EPSCs in hippocampal CA1 pyramidal cells, without altering their amplitude, suggesting that PKC activation leads to an increase in transmitter release.**

**The PE stimulation of glutamate release was attenuated approximately 50% by application of nifedipine. Based on our earlier findings that phorbol ester application causes L-type calcium channels to open at negative potentials, we hypothesized that a PE-stimulated increase in resting membrane conductance activity may underlie part of the PE stimulation of glutamate release. If PE-induced and tetanic stimulation-induced potentiation share a common expression mechanism, then application of nifedipine should not reduce tetanus-induced LTP expression. We have examined the effects of nifedipine on the expression of LTP. Our preliminary results suggest that nifedipine (10 μM), 20 min after LTP induction, may reduce LTP by up to 20%, without a significant decline in basal transmission. In contrast, nifedipine 60 minutes after induction of LTP had no apparent effect on the magnitude of potentiation. We are currently pursuing experiments to show if presynaptic L-type calcium channels play a role in the expression of LTP.**

628.8

**EFFECTS OF NIFEDIPINE ON LONG-TERM POTENTIATION IN AREA CA1.**


**We investigated whether changes in transients or residual levels of presynaptic calcium could be responsible for the maintenance of long-term potentiation (LTP) in area CA1 of hippocampal slices of guinea-pig. A novel technique for electrically evoking field responses in rat hippocampal slices (Ari, 1991; Nature 349, 67-69). In this study we have extended these observations to hippocampal slices of guinea-pig, cats, rats, mice, and rabbits.**

**Brief application of a K+ channel blocker to CA1 neurones of the in vitro rat hippocampal slice has been reported to induce a long lasting (TEA, MCDP) or decremental (4-AP) potentiation of synaptic responses (Boyett & Ben-Ari, 1991; Nature 349, 67-69). In this study we have extended these observations to include dento-hippocampal (Dx) slices homogenised from the cortex of green and black marmoset, which are points and sex-specific blocks of voltage-activated K+ currents.**

**Extractable field potential responses were recorded from the CA1 region of hippocampal slices using conventional methods and solutions. Potentials were evoked by constant stimulation of the Schaffer collaterals at 0.1 Hz/0.02ms duration, at 30°C. Drugs were applied in the perfusing medium for a period of ten minutes, slices would then be returned to the baseline condition.**

4-AP (10 mM), Toxin I (100 mM) and TEA (25mM) induced repetitive firing and increased the amplitude of the first population spike by 165%, 24% and 23% respectively, immediately following a ten minute exposure (n=5 in each case). The effect of 4-AP was rapid in onset but slowly reversed during washout. The enhancement due to TEA (peak ca 225% at 15 min wash) was slightly slower in onset and was poorly reversible (ca 150% enhancement at 60 min wash). Slices treated with Toxin I exhibited a slowly developing persistent potentiation which peaked 15 min after removal of the toxin (100% enhancement at 60 min wash). Although MCDP (1uM), γ-Dxs (60mM) and 8-Dxs (10mM) did not induce multiple population spikes (n=3 for each drug), MCDP promoted a rapid and persistent enhancement of the population spike amplitude (ca 200% at 60 min wash) and 8-Dxs produced a slowly developing enhancement taking up to 60 min to reach a maximum (ca 100% enhancement). γ-Dxs had similar actions as 8-Dxs but was much less potent (ca 40% enhancement at 60 min wash).

The qualitative differences in activity of the toxins may reflect relative selectivities for pre- and post-synaptic elements.
HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) SELECTIVELY MODIFIES THE BINDING PROPERTIES OF GLUTAMATE RECEPTORS.


Neurosciences Program, University of California, Irvine, CA 92697.

Several lines of evidence indicate that LTP is associated with a change in some properties of postsynaptic glutamate receptors. In the present study we have used quantitative autoradiography of radiolabeled ligands selective for the AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) and NMDA (N-methyl-D-aspartate) subtypes of glutamate receptors to examine the binding properties of glutamate receptors in frozen brain sections obtained from rats in which perofrnat path LTP was induced. Eleven adult male Long-Evans rats were anesthetized and implanted with a stimulating electrode in the perforant path and a recording electrode in the dentate hilus. LTP was induced by delivering either two 25 400 Hz bursts separated by 300 msec (n = 6) or ten 25 400 Hz bursts separated by 10 sec (n = 5) at a current intensity sufficient to elicit a 1-2 mV population spike. One hour following stimulation the rats were sacrificed and their brains rapidly dissected and frozen.

Perforant path LTP induction resulted in a selective increase in [H]--AMP binding in the molecular layer of the dentate gyrus ipsilateral to the perforant path stimulation as compared to the contralateral side. The increase in AMPA binding in the dentate gyrus was highly correlated (r = .93, p < .0002) with the LTP-induced change in EPSP slope recorded in this structure one hour after LTP induction. No changes in the binding of either [H]--CNQX (6-nitro-7-sulfamoylbenzofuro[3,2-d]) of kainate, an antagonist of the AMPA receptor, or [H]--TCP (N-(1-thiophenyl)cyclohexyl) piperidines, a ligand for the NMDA receptor/channel, were observed. Together, these results indicate that a modification in postsynaptic AMPA receptors plays a critical role in the expression of synaptic enhancement following LTP induction in the hippocampus. Supported by NIH (AG01347) and the McKnight Foundation to RFT and NSF (96284) to MB.
628.18 THE GENERATION OF AN cDNA LIBRARY FROM THE MOSSY-FIBER-CA3 SYNAPSE
D.T. Rivera, B.E. Derrick and J.L. Martinez, Jr., Department of Psychology, University of California, Berkeley, CA 94720.
Long-term synaptic changes (e.g. long-term potentiation, LTP) are essential for complex behavior and memory. Previous studies have suggested that induction of LTP involves the activation of specific glutamate receptors. We have generated a cDNA library from the mossy fiber-CA3 synapse in an effort to identify these receptors. The cDNA library will be characterized in various ways including subtraction of cDNAs from the non-tetanized contralateral hippocampus of the same rat. Supported by NIDA #DA04195; NSF DIR-910195; The Rennie Fund.

628.19 NONLINEAR SUMMATION OF NMDA RESPONSES TO BURST STIMULATION IN PIRIFORM CORTEX SLICES REQUIRES GABA\textsubscript{A} RECEPTOR BLOCKADE. A. Kaga* E.D. Kandel and L. Haberly, Neurosciences Training Program and Department of Anatomy, Univ. of Wisconsin, Madison, WI 53706.
As previously reported (Kanter and Haberly, Soc Neurosci Abstr. 17:385, 1991), associative LTP in piriform cortex can be induced only after blockade of GABA\textsubscript{A} (but not GABA\textsubscript{B}) receptors. We are now investigating the role of the interaction of GABA-mediated and NMDA-mediated responses in the regulation of synaptic plasticity. Intra- and extracellular recordings were made from layer II pyramidal cells in piriform cortex slices. In the presence of the non-NMDA glutamate receptor antagonist DNQX (20 μM), stimulation of either afferent or association fibers evoked responses consisting of a fast, GABA-mediated IPSP (depressing at firing membrane potential), an NMDA-mediated EPSP and a slow, GABA\textsubscript{A}-mediated IPSP, as revealed by specific receptor blockers. Responses to bursts of 2-6 pulses were obtained in DNQX with or without the GABA\textsubscript{A} antagonist bicuculline methiodide (10 μM). After GABA\textsubscript{A} blockade the NMDA-mediated responses to bursts were greatly enhanced, exhibiting a nonlinear summation. This phenomenon was observed from stimulation of both afferent fibers (layer Ia) and association fibers (layer Ib or layer III) and was optimal observed at stimulation frequencies of 100 to 200 Hz (320 Hz). Bursts with inhibition intact produced at best a linear summation of the depolarizing response (GABA\textsubscript{A}-mediated IPSP + NMDA-mediated EPSP). In some cells the size of the burst response after GABA\textsubscript{A} blockade was several fold larger than with GABA\textsubscript{A} inhibition intact. Blockade of the slow IPSP with the GABA\textsubscript{A} antagonist CGP-35348 (1μM) did not by itself enhance NMDA responses to bursts but did shift the optimal stimulus frequency for the enhancement produced by GABA\textsubscript{A} blockade. Supported by grant NS19665 to L.B.H.

In a previous study on behaving rats, we reported that patterned primed burst (PB) stimulation of the contralateral CA1 or CA3 resulted in robust long-term potentiation (LTP) at the basolateral dendritic synapse of CA1, but only weak or no LTP at the apical dendritic synapses (Leung et al., Neurosci. 48:63,1992). This abstract reports factors that may enhance the apical dendritic LTP in behaving rats. Septal co-stimulation, at an intensity which caused desynchronization of the hippocampal EEG, or 50 mg/kg i.p. atropine sulfate did not significantly enhance the success of PB-induced LTP. High frequency (HF) trains of 100-200 Hz, at 3x threshold intensity, were successful in eliciting LTP in 8 of 10 rats, a significantly higher success rate than PBs of a similar intensity in the same rats (0/3). HF stimulation, however, almost always elicited afterdischarges and postictal depression. The mean apical dendritic LTP peaked at about 113% (of baseline) at 2 hr post-injury.
The non-decremental time course and low magnitude of the apical dendritic LTP is clearly different from the decremental, high-amplitude basolateral dendritic LTP in CA1. Furthermore, the N-methyl-D-aspartate antagonist APV did not significantly change the HF-induced apical dendritic LTP in 5 rats, while it strongly attenuated the basolateral dendritic LTP induced by HF or PB (Leung and Shen, Neurosci. Abstr. 17:511). The time course, magnitude and HF-dependency of the apical dendritic LTP are consistent with the properties of an APV-resistant LTP in vitro (Grover and Teyler, Nature 347:477). However, in vitro, the propensity and properties of LTP appear to be the same at the basal and apical dendrites in CA1. Isolateral stimulation and recording in CA1, placed as in an in vitro slice, was done in 16 behaving rats. The results corroborated those using contralateral hippocampal activation, in that LTP was robust at the basal but not the apical dendrites. (Supported by NSERC.)

628.21 K*-INDUCED FACILITATION OF HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) MAY BE MEDIATED THROUGH AN ACTION ON NMDA RECEPTORS. B. Ballyk, Program in Molecular Physiology, University of California at San Diego, La Jolla, CA 92037.
In the hippocampus, repetitive activation of CA, afferents, which induces LTP, results in elevated extracellular K* concentrations. We have shown that elevating extracellular K* during a weak tetanus either by bath application or by iontophoresis at the CA, synaptic zone facilitates the induction of LTP. We hypothesize that this facilitation is through an action on the NMDA receptor. Dendritic population EPSPs were recorded in the CA, region of gerbil hippocampal slices by stimulation of stratum radiatum. Weak EPSPs (<300 μV) failed to exhibit LTP following a 1000 Hz, 1 sec tetanus (104±3% (SEM) of control, 30min post tetanus, n=6). K* iontophoresis applied at the site of recording for 10s, did not in itself produce long-lasting effects on EPSPs (96±2% of control, 30min post K*, n=6). In the presence of 25μM d-s-camphor-3-sulfonic acid, delivery of the weak tetanus with simultaneous iontophoresis of 300mA K* application of K* in the dendrites did not result in significant potentiation of the EPSP (95±4% of control, 30min post K* & tetanus, n=6). However, following washout of the NMDA receptor antagonist, the combination of weak tetanus and K* resulted in significant potentiation of EPSPs (138±6% of control, 30min post K* & tetanus, n=6). In other experiments, intracellular recordings were obtained from CA, pyramidal neurons, and responses to NMDA, applied iontophoretically at the dendrites, recorded. Increasing both the intensity and the duration of the NMDA response by 85% increased K* response by 85% (n=3). We suggest that elevated extracellular K* at the synaptic zone resulting from an afferent tetanus enhances NMDA receptor activation required for successful induction of LTP. Supported by the MRC (Canada). BAB is a Queen’s Graduate Fellow.

The plasticity of synaptic input to the septum was investigated in an in vivo slice preparation. Septal neurons were recorded intracellularly and labelled with bicysitin. An extracellular stimulating electrode was placed in the medial septum of coronal slices and a recording electrode was placed about 1 mm lateral to the stimulating electrode. Extracellular stimulation evoked a rapid EPSP followed by an IPSP that lasted 50-200 msec. Addition of the GABA, receptor antagonist bicuculline (BIC) (30 μM) revealed an EPSP that lasted 40-80 msec and reversed in polarity near 0 mV. The EPSP was blocked by kynurenic acid (2 mM) indicating that excitatory amino acids mediate this synaptic potential. We next investigated plasticity of the EPSP using two separate paradigms, both in the presence of BIC. First, the slices were briefly (5-10 min) exposed to 100-nM kainate. This caused a potentiation of the EPSP that lasted for 30-50 min. The second paradigm consisted of high frequency (HF)-induced LTP. HF stimulation of afferents to determine whether excitatory synaptic input to the septum could express long-term forms of use-dependent synaptic plasticity. A population of cells that received the tetanus in the presence of BIC expressed a long-term enhancement of the EPSP that lasted for 30 min. This study was funded by a grant from the American Federation for Aging Research (AFAR).
628.23
FREQUENCY-DEPENDENT ENHANCEMENT OF MONOSYNAPTIC INHIBITORY POSTSYNAPTIC EVENTS IN THE RAT NUCLEUS TRACTUS SOLITARIUS (NTS).

Whole cell patch recordings in the NTS were made in transverse slices of rat brainstem (age 2-6 weeks). Postsynaptic potentials and currents were evoked by low frequency (0.2Hz), electrical stimulation in the area of the tractus solitarius. Since most NTS neurons received a mixed excitatory and inhibitory input, inhibitory postsynaptic potentials (IPSPs) and currents (IPSCs) were studied in isolation by applying blockers of excitatory amino acid receptors. CNQX or DNQX completely blocked excitatory synaptic responses in most neurons but in some cases APV or AP-5 were also applied to block a residual excitatory component. The inhibitory synaptic events were completely blocked by bicuculline. The presence of a GABA_A-mediated IPSP was obtained although baclofen had both pre- and postsynaptic effects.

IPSPs and IPSCs were studied at potentials of -50 to -60mV during and after stimulation at higher frequencies (1-20Hz). In 8 out of 12 cases the IPSC or IPSP was increased in amplitude after stimulation at frequencies of 5Hz or higher. Depression of the inhibitory event after the higher frequency stimulation was not observed. The potentiation was long-lasting (up to 1 hour) and could be evoked more than once during a recording. The reversal potential of the inhibitory IPSP/IPSC was not altered after potentiation.

The induction and maintenance of the potentiation was resistant to blockade of NMDA receptors with AP-5 and in addition was not affected by the GABA_A antagonist 2-Oh-saclofen.

Supported by MRC, Wellcome Trust & NIH.

628.25
ACTIVITY-DEPENDENT PLASTICITY OF INHIBITION IN THE DENTATE COMMISSURAL PATHWAY: RELATIONSHIP TO EPSP/SPIKE DISINTEGRATION.S.D. Tupsap*, O. Steward, J.J. Ramirez, W.B. Levy. Dept. of Neuroscience, University of Virginia School of Medicine, Charlottesville, VA 22908.

We tested the hypothesis that a decrease in feed-forward inhibition contributes to LTP-associated EPSP/spike (E-S) dissociation in the hippocampus. Because the dentate commissural pathway (CP) and the perforant path (PP) activate a common pool of interneurons, a change of synaptic efficacy due to activation of one input could induce changes in the other.

In urethane-anesthetized rats we measured the inhibition of PP population spikes by the CP at intracellular potentials of 6 and 12 mV. This measure and an E-S function were obtained before and after a) PP tetany (400 Hz, 16 msec) and b) CP tetany (200 Hz, 30 msec).

Low intensity PP conditioning (spike the PP) resulted in the measures of CP inhibition and large E-S shifts to the left; higher intensity PP tetany produced increases in CP inhibition and smaller leftward E-S shifts. CP conditioning induced no changes in CP inhibition and variable E-S shifts.

Post-hoc analysis of the CP-tetany cases revealed that leftward E-S shifts accompanied depression of the PP (EPSP to 0%) and right E-S shifts accompanied potentiation of the PP (EPSP to 2%).

The inhibitory circuit of the dentate gyrus expresses activity-dependent plasticity under conditions which alter the E-S relationship. This suggests that changes in inhibition contribute to EPSP/spike dissociation. The pattern of changes is best explained by independent plasticity of excitatory and inhibitory synapses. Supported by K08NSO1430 to RT and BNS 8617806 to OS. WBL was supported by NIH 54648 and NIMH 46161.

628.26
A DEVELOPMENTAL ANALYSIS OF LONG-TERM POTENTIATION IN THE FREELY-MOVING RAT.

The ability of rats to establish and support LTP of the perforant path/dentate granule cell synapse was examined at 15, 30 and 90 days of age. Hippocampal dentate granule cell field potentials were recorded, before, and at 60 min post potentiation, and at 24 hrs after potentiation of the perforant path. Wavesforms were analyzed for changes in population EPSP slope (a measure of synaptic drive) and population spike amplitude (a measure of population discharge). 90-day old animals exhibited an 18% enhancement of the population EPSP slope 30 mins after potentiation, rising to approximately 40% above baseline 5 hrs. after potentiation and remaining at this level at 24 hrs. In contrast, EPSP slope measures obtained from 30-day olds declined nearly 25% from baseline 30 mins after potentiation, falling to regain baseline over the 24 hr test period. Population spike amplitude measures in the 30-day old group enhanced beginning 1 hr post-potentiation. This measure rose to >100% after 24 hrs. The rate of population spike enhancement obtained from 30-day old animals closely paralleled that of the 90-day old group, however, 90-day old animals consistently attained levels of enhancement 20% higher than those in the 30-day old group. Preliminary results in 15-day olds also indicate potentiation of the population spike component in association with decreases in EPSP slope measures. The results indicate a dissociation between the EPSP slope and population spike components in younger animals, which may reflect functional immaturity of transmitter systems modulating dentate granule cell excitability. Supported by NSF Grant #BCS9010616.
LONG-TERM POTENTIATION V

628.29

REVERSIBLE INACTIVATION OF THE LOCUS COERULEUS, BUT NOT THE MEDULLARY SEPTUM, PREVENTS HIPPOCAMPAL LONG-TERM POTENTIATION. E.J. Barac, S.E. Krahl, and D.C. Smith. Department of Psychology and School of Medicine, Southern Illinois University, Carbondale, Illinois 62901.

Several studies have suggested that norepinephrine (NE) may play an important modulatory role in the induction of long-term potentiation (LTP) in the dentate gyrus (DG) of the hippocampus (cf. Neuman & Harley, Brain Res, 273:163, 1983). The present study investigated whether the microinfusion of 2% lidocaine hydrochloride, a reversible local anesthetic, into the locus coeruleus (LC) prevents the induction of DG-LTP in the in situ, LC-anesthetized, adult male rat.

Long Evans rats were implanted with a unilateral guide cannula immediately above the LC or medullar septum (MS), and with an electrode in the ipsilateral DG from which EPSPs, evoked by 80-300 μA biphasic square-wave pulses from a perforant path (PP) bipolar stimulating electrode, were recorded. Following a 1-hr stabilization period, both groups were microinfused with 0.5 μl of saline or lidocaine over a 2-min period. Eight minutes later, the animals received high-frequency stimulation (6 trains of 8 pulses at 400 Hz) of the PP.

Saline-infused controls displayed significant EPSP potentiation following high-frequency stimulation in both the LC, F(1,9)=5.4, p<.05, and MS groups, F(1,7)=10.5, p<.05. In contrast, lidocaine infused into the LC prevented EPSP potentiation when compared to pre-potentiation levels, F(1,7)=1.2, p>.05, or saline-infused potentiated controls, F(1,26)=3.98, p<.05. Lidocaine infused into the MS did not alter EPSP potentiation as compared to saline-infused controls, F(1,24)=0.0, p>.05. These findings suggest that the release of NE from the locus coeruleus is necessary for the induction of DG-LTP.

628.31

NMDA-ACTIVATED CONDUCTANCES PROVIDE SHORT-TERM MEMORY FOR DENDRITIC SPINE LOGIC COMPUTATIONS. Reddick V. Jensen and Gordon M. Shepherd. (Section of Neurobiology Yale University School of Medicine, New Haven, CT 06510) (+Department of Physics, Wesleyan University, Middletown, CT 06457)

Active conductances in or near dendritic spines may permit elaborate computational processing of multiple synaptic inputs long before these signals reach the soma. Numerical models of dendritic trees indicate that the interaction of postsynaptic potentials in active spines can generate simple logic operations such as AND, OR and NAND gates. However, because the spine head EPSP's closely follow the underlying, short-duration (1-5 ms), synaptic conductances, previous studies concluded that precise timing of synaptic inputs would be critical for these logic operations to occur. We show that this temporal limitation on dendritic computation can be relaxed by the inclusion of slow (100-500 ms), voltage-dependent, NMDA-receptor mediated conductances in the spine heads. Our numerical simulations show that this simple mechanism provides a short term memory (=100 ms) for logic AND gates with time-delayed inputs on one or more spines.

Supported by ONR and NIMHD.

628.30


Recently our laboratory has found that several pharmacological treatments which produce LTP-like synaptic enhancement also produce increases in synaptic phosphorylation. Greengard's group has shown that synapsin I may play a role in the increased transmitter release seen in LTP. A critical test of this hypothesis is to determine whether synapsin phosphorylation is correlated with LTP induced by classical means, i.e. tetanic stimulation. Unfortunately, tetany delivered via a single electrode induces LTP in only small fraction of the synapses in a slice, resulting in signal detection problems in biochemical studies.

To address this problem we have begun using a mini-slice preparation in which the CA1 region is microdissected from a typical hippocampal slice. In addition, we have also begun using an electrode array (Longreach Scientific Resources, Ont.'s Island, ME) consisting of four monopolar stimulating electrodes placed side by side and spaced 100 microns apart. When the stimulating electrodes are separated in the array by 150 microns or less, such electrodes can be paired to produce paired pulse facilitation when a 50 msec inter-stimulus interval is used. Moreover, tetanic stimulation delivered to one of the electrodes produced LTP in the adjacent unstimulated electrode in 13 of 19 experiments. These effects can be seen in stratum radiatum when the electrode is positioned either parallel or perpendicular to the CA1 cell layer. We have examined the biochemical effects of synchronous tetany (400 Hz for 1 sec) delivered to all four electrodes at two positions in the stratum radiatum of a CA1 mini-slice. Such stimulation produced an increase in synapsin I phosphorylation at its CAM kinase II sites in 7 of 10 experiments.

628.32

HOMOSYNAPTIC LONG-TERM DEPRESSION IN CA1 IN VITRO: DEVELOPMENT, MECHANISM, AND INTERACTION WITH LTP. S.M. Dudek* and M.F. Bear. Center for Neural Science, Brown University, Providence, RI 02912.

Last year, we showed that 900 pulses delivered to the Schaffer collaterals at 1-3 Hz consistently yielded a depression of the CA1 population EPSP that persisted without signs of recovery for > 1 hour following cessation of the conditioning stimulation. This long-term depression (LTD) was specific to the conditioned input, ruling out generalized changes in postsynaptic responsiveness or excitability. LTD was dependent on the stimulation frequency; 900 pulses at 10 Hz caused no lasting change, and at 10 Hz a synaptic potentiation was usually observed. This, coupled with the observation that the depressed synapses continued to support long-term potentiation in response to a high frequency tetanus, suggested that LTD is accounted for by a modification of synaptic effectiveness rather than damage to or fatigue of the stimulated inputs.

We have extended these results in three ways. First, we have found that AP5 blocks induction of LTD. Because the release of neurotransmitter at the Schaffer collateral - CA1 synapse is not directly affected by AP5, this observation suggests that depletion of neurotransmitter is not a likely explanation for LTD. Moreover, our data suggest that synaptic depression can be triggered by prolonged NMDA receptor activation that is below the threshold for inducing synaptic potentiation. Second, we have found that LTD in the CA1, if of sufficient magnitude during early postnatal development, Thun, we have evidence that LTD may be "unmasked" by LTD, suggesting that the site of modification for LTP and LTD is the same. We propose that this mechanism is important - perhaps as important as LTP - for the modifications of hippocampal response properties that underlie some forms of learning and memory.

(Supported by ONR Young Investigator Award to M.F.B.)

628.33

COMMON FORMS OF PLASTICITY IN HIPPOCAMPUS AND VISUAL CORTEX IN VITRO. A. Kirkwood, C.D. Aluisen and M.F. Beart. Department of Neuroscience, Brown University, Providence, RI 02912.

The best studied model for synaptic plasticity in vitro is the CA1 region of adult rat hippocampus, where both homosynaptic long-term potentiation (LTP) and homosynaptic long-term depression (LTD) have been shown to occur. Using a novel stimulation-recording arrangement in visual cortex, we have found that remarkably similar forms of plasticity can be elicited with precisely the same types of signals that are effective in hippocampus.

Recent work in this laboratory has shown that homosynaptic LTD can be induced by low frequency stimulation (LFS) in CA1. Similarly, depression of the layer V-IV synaptic connections can be induced by LFS (900 pulses at 1 Hz). The average magnitude of the LTD was 15 ± 3% (n = 11). In contrast, the synapses produced no change (±3 ±10%, n=5).

The combined results suggest that hippocampus should no longer be considered a privileged site for synaptic plasticity in the adult brain. Even the mature primary visual cortex can exhibit plasticity of comparable magnitude and robustness.

Supported in part by the NEI, ONR, and the HFSP.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992

The magnocellular cholesteric neurons of the rat basal forebrain are known to be rich in neurotensin (NT) receptors. The whole-cell clamp method was used to investigate the effect of NT on dissociated cultured neurons from the nucleus basalis (NB) of newborn rats. Membrane potential was held at -74mV and returning depolarizing (20mV, 100ms) and hyperpolarizing (50mV, 100ms) voltage steps. Application of NT (1µM) produced a long-lasting decline of conductance (154% for 10mM recovery, n=5) together with a slow inward current, reflecting cellular excitatory. This NT sensitive current reversed inwardly directed rectification with a reversal potential approximately equal to or more negative than EK. Occasionally the reversal potential was substantially more negative than EK, suggesting the concomitant activation of a non-selective ion channel. In cells preloaded with the dye hydrolysis resistant GTP analog GTP-γS, application of NT produced an almost irreversible reduction in membrane conductance and inward shift of the base-line current. In SB cultures pretreated with pertussis toxin (500ng/ml, 15-22 hrs) the NT effect was not decreased from controls. These findings suggest that the conductance decrease produced by NT is mediated through a pertussis toxin-sensitive G protein. Supported by PHS grants AG06993 and I3P30810167.


We have performed cell-attached patch-clamp recordings on freshly dissociated rat corpus striatum (caudate and putamen) neurons in order to characterize the different types of K+ channels present, and to study the extent of these channels modulated by dopamine receptors. As previously described, an 85 pS channel was observed when the D2/g a agonist quinpirole was present in the patch pipette, but was not observed in the absence of drug, or when agonist was applied via a macropipette to the cell body. Two other classes of channels were also observed near resting membrane potential with 140 mM KCl in the patch pipette. Two others were inwardly rectifying K+ channels with conductances between 8-30 pS. Secondly, there were voltage-sensitive K+ channels of 100-200 pS, which appeared to be large conductance Ca2+-activated K+ channels. Neither of these two types appeared to be modulated by dopaminergic drugs. These results may help clarify which subtypes of K+ conductances are involved in the dopamine stimulated response. (Supported by the Pharmaceutical Manufacturers Association, Tourette Syndrome Association, and NIH FIRST award MH-48545).

629.3 MODULATION OF AN OUTWARD MEMBRANE CURRENT BY OXYGEN IN RAT PHEOCHROMOCYTOMA (PC12) CELLS. W. H. Zhu * M. J. Burns, M.F. Cowey-King, A. Lawyer and D. H Millborn. University of North Carolina, Chapel Hill, NC.

PC12 cells are dopamine secreting cells that are morphologically and chemically similar to the 110 mS constituent of mammalian carotid body. Type I cells depolarize and release dopamine when exposed to low O2 (hypoxia). It was reported recently that depolarization of type I cells occurs as a result of a decrease in conductance of a O2 sensitive potassium channel (Science 241,1988). However, the actual mechanism by which type I cells detect low O2 and transduce this signal into altered cellular function remains unknown. The present study was undertaken to determine if PC12 cells respond to hypoxia in a manner similar to type I cells. If so, this cell line might prove valuable for studying fundamental mechanisms associated with O2 detection and signal transduction. Whole-cell recordings were performed on rat PC12 cells exposed to control gas (hypoxia: 95% O2, 5% CO2, ambient air, 21% O2) or hypoxia (<10% O2). Membrane potential was voltage clamped at -40mV and change in current was measured in response to positive voltage steps (20mV, 30ms) from -80mV to +80mV. We measured a voltage sensitive outward current that was maximum (800-900 pA) at the largest voltage step. The magnitude of this outward current was decreased substantially when cells were exposed to hypoxia or hyperpolarized and remained constant when reexposed to control gas. Current-voltage plots showed that this outward current is voltage-sensitive and has reversal potential of about 60mV. These findings suggest that the oxygen-sensitive current in PC12 cells is a voltage-dependent potassium current. In addition, we measured dopamine concentration in media of cells exposed to control gas and found that hypoxia evoked a 2-3 fold increase in dopamine release from PC12.


The GH3 cell line serves as a model system to study the cellular control of TRH-induced prolactin secretion. One difference between this cell line and normal lactotrophs is the lack of dopamine (DA) receptors in GH3 cells. Recently, the expression of DA receptors in GH3 cells induced by treatment with epidermal growth factor (EGF) was shown by binding studies (Missale et al., Endocrinol 128, 1991). In normal rat lactotrophs, DA induces a transient hyperpolarization and stop of action potentials (Israel et al., Chloride currents in mammalian neocortical neurons, J. Physiol. 390, 1987). In GH3/B6 control cells (where cell patch clamp), no changes in the electrical activity could be induced by the application of 5 µM DA. After prolonged (>4 d) treatment with 100 nM EGF and 100 µM progesterone, the GH3/B6 cells consistently responded to DA with a decrease in frequency of action potentials, and an increase in action potential duration leading to more hyperpolarized afterpotentials. This effect lasted several minutes. D2 receptors present in rat lactotrophs are shown to be coupled to pertussis toxin (PTX)-sensitive G-proteins (Lledo et al., Brain Res. 558, 1991). In EGF-treated GH3/B6 cells, the DA response was not inhibited by preincubation of the cells with PTX (500 µg/ml, 5b).

Our results show that EGF treatment resulted in the functional expression of DA receptors in GH3/B6 cells, but differences in electrophysiological response and PTX-sensitivity suggest that these DA receptors are not identical to those of normal rat lactotroph cells.

629.5 MODULATION OF AN OUTWARD MEMBRANE CURRENT CAUSED BY OXYGEN IN RAT PHEOCHROMOCYTOMA (PC12) CELLS. W. H. Zhu. * M. J. Burns, M.F. Cowey-King, A. Lawyer and D. H Millborn. University of North Carolina, Chapel Hill, NC.

PC12 cells are dopamine secreting cells that are morphologically and chemically similar to the 110 mS constituent of mammalian carotid body. Type I cells depolarize and release dopamine when exposed to low O2 (hypoxia). It was reported recently that depolarization of type I cells occurs as a result of a decrease in conductance of a O2 sensitive potassium channel (Science 241,1988). However, the actual mechanism by which type I cells detect low O2 and transduce this signal into altered cellular function remains unknown. The present study was undertaken to determine if PC12 cells respond to hypoxia in a manner similar to type I cells. If so, this cell line might prove valuable for studying fundamental mechanisms associated with O2 detection and signal transduction. Whole-cell recordings were performed on rat PC12 cells exposed to control gas (hypoxia: 95% O2, 5% CO2, ambient air, 21% O2) or hypoxia (<10% O2). Membrane potential was voltage clamped at -40mV and current change was measured in response to positive voltage steps (20mV, 30ms) from -80mV to +80mV. We measured a voltage sensitive outward current that was maximum (800-900 pA) at the largest voltage step. The magnitude of this outward current was decreased substantially when cells were exposed to hypoxia or hyperpolarized and remained constant when reexposed to control gas. Current-voltage plots showed that this outward current is voltage-sensitive and has reversal potential of about 60mV. These findings suggest that the oxygen-sensitive current in PC12 cells is a voltage-dependent potassium current. In addition, we measured dopamine concentration in media of cells exposed to control gas and found that hypoxia evoked a 2-3 fold increase in dopamine release from PC12.

629.6 MEMBRANE POTENTIAL CHANGES INDUCED BY ANOXIA IN RAT DORSAL VAGAL MOTONEURONES ARE INFLUENCED BY INTRACELLULAR pH. A. L. Cowan, P. L. Martin and J. B. Redman. Division of Neuroscience, JSMIR, and Division of Botany and Zoology, Australian National University, Canberra, A.C.T. 2601, Australia.

This study was undertaken to investigate the influence of intracellular pH (pHi) on the membrane potential changes induced by anoxia. Intracellular recording from dorsal vagal motoneurones (DVMs) in brainstem slices prepared from rats aged 35-45 days demonstrated that 44% of DVMs hyperpolarized and 45% depolarized during anoxia in bicarbonate/CO2 buffered artificial cerebrospinal fluid (ACSF). However, in 2- hydroxyethylpiperazine-N,N'-2-ethanesulfonic acid (HEPES) buffered ACSF (which is expected to cause an increase in intracellular pH (pH)), anoxia resulted in a depolarization of 9.5±1.2 mV (SEM) in all 31 neurones tested. There was an increase in input resistance of 24.3±3.1% in 42% of the neurones and a decrease in input resistance of 12.5±1.1% in the remainder. The effects persisted when spike dependent synaptic transmission was blocked with tetrodotoxin. Addition of tetrathylammonium chloride, 4-aminopyridine, tetrodotoxin, ouabain or manganese, singly or in combination, showed that the membrane potential changes involve an increase in a calcium current which is counteracted to some extent by a small increase in the delayed rectifier current. A residual depolarization associated with an increase in input resistance is not due to an effect on the A current or on chloride channels. Inhibition of the Na-K ATPase did not appear to be involved. These results contrast with the effects of anoxia on membrane potential in bicarbonate/CO2 buffered ACSF (pH) in which pHi is presumed to be lower. It is concluded that changes in pHi of neurones during anoxia may be responsible for the early changes in their electrical properties. 1. Gaillard, S. & Dupont, J. L. (1989) J. Physiol. 417, 79-83. 2. Cowan, A. L. & Martin, P. L. (1992) J. Physiol. (In Press).
629.7 ANOXIC CHANGES IN MEMBRANE NOISE OF HIPPOCAMPAL NEURONS. M. Glavnikov, P. Miu and K. Knjizevic*. Anesthesia Research Dept., McGill University, Montreal, Que, H3G 1Y6 Canada. Brief periods of anoxia elicited a characteristic hyperpolarization of pyramidal cells (Hansen et al. 1982, Acta Physiolog. scand. 115: 301). To obtain further information on the nature of the underlying conductive changes, we recorded in CA1 neurons, by single electrode voltage-clamp, the currents and (at higher gain) the changes in electrical noise generated by anoxia (2-3 min of 95% N₂ - CO₂), in the presence of TTX. The experiments were done in slices (from Sprague-Dawley rats) kept at 37°C.

In the majority of cells, the anoxic outward currents seen at holding potentials (V₉0) between -70 and -30 mV were associated with an increase in variance (σ²) of the base-line noise. This indicates the opening of ionic channels, in keeping with the observed simultaneous increase in macroscopic conductance. Also, in keeping with the macroscopic currents, the unitary currents reversed from outward to inward at -78 ± 6.6 mV (n=3; mean ± SD). The corresponding single channel conductance varied over a wide range: 20.2 ± 16.4 pS.

In two cells, the anoxic outward current was accompanied by a reduction in electrical noise. This inverse relation between σ² and current confirms previous evidence that anoxia can elicit both opening and closing of certain ionic channels.

Supported by the Canadian Medical Research Council.


During astrocytic swelling caused by extracellular hypotonic or high K⁺ solutions non-selective cation channels, so-called Cl-/K⁺ channels, are opened readily in astrocytes when hypotonic rather than isoosmotic recording solutions are used. To study the pattern-dependency of the increased anion conductance we have recorded the activity of single astrocytes in culture using the patch-clamp technique. With symmetrical and non-symmetrical CI⁻ concentrations in the pipette and both the channels in excised inside-out patches showed the highest probability to open at or near zero membrane potential, thus indicating that membrane depolarization induces the channel to open. The activated Cl⁻ channel is able to attain at least five discrete open sublevels. When the maximal open state is reached the channel has the tendency to stay open for seconds and then closes abruptly in one step. Opening of all the sublevels leaves the channel in an unstable state which is characterized by fast flickering openings and closings. The channel is blocked by the anion transport inhibitor L-444,711, which also blocks RVD.

Such a channel may function to let Cl⁻ out during RVD. To verify the existence of these channels in other than cultured mammalian astrocytes we are also using cortical tissue print cultures. The role of these effects in the mediation of ethanol intoxication remains unknown.

We have recently characterized the sequence of events coupling muscarinic receptor activation to the potentiation of the delayed rectifier potassium current (IK) in rat hippocampal CA1 neurons. Ig was recorded from mature rat CA1 neurons in basic slices using the whole-cell patch-clamp method. The currents were recorded during periods of 20 μM carbachol reversibly potentiated Ig (mean=80%) with an onset of action of 1-3 minutes. A reversible decrease in holding current was not associated with changes in the resting conductance was also observed during carbachol application. The intracellular mediators of this enhancement were shown to include a G protein, IP3, DAG, and PKC. This kinase-modulated current was used as an assay to characterize possible actions of ethanol on second messenger systems. Both application of 20 mM ethanol also enhanced Ig with a slower onset of action and recovery rate than carbachol and no associated change in holding current. In addition, incubation of slices with 20 mM ethanol for 10-25 minutes did not consistently inhibit subsequent carbachol enhancement of Ig. Ethanol pretreatment did however reduce the carbachol-induced reduction in holding current.

These preliminary results demonstrate that ethanol, at a clinically relevant concentration, can potentiate Ig in central mammalian neurons. The cellular mechanisms underlying this enhancement are currently under investigation.

Supported by the MRC and an Ontario Graduate Scholarship.

629.9 GIAL MODULATION OF TRANSIENT POTASSIUM CURRENT EXPRESSION IN CULTURED MUSCLE HIPPOCAMPAL NEURONS. R-J. Wu* and M. Barish. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Rodent hippocampal neurons express multiple transient potassium currents that influence action potential duration and accommodation to sustained depolarization. We have studied pyramidal-shaped neurons in dissociated cell cultures prepared from mouse hippocampus on embryonic days 15-16 using whole-cell gigaohm-seal voltage clamp techniques. These neurons exhibit two transient potassium currents, termed A- and D-currents, that can be separated based on steady state inactivation characteristics and sensitivity to 4-aminoipyridine (Wu and Barish, J. Neurosci., in press). We have observed that after 5-7 days in culture A-current density (current normalized to membrane area) is larger than D-current density in neurons growing on glial cells (primarily GFAP-immunoreactive astrocytes), while D-current density is larger in neurons growing only on the poly-lysine/laminin-coated glass substrate. This variation is approximately reciprocal; the sum of A- and D-current densities is similar in both types of neurons. The glia-derived signal probably acts by cell surface interaction or restricted diffusion of a soluble factor, as neighboring cells show differences in potassium current expression based on glial contact. Living glia may be required, as currents in neurons growing on dried glial membranes or methanol-fixed glia resemble those in neurons that do not touch glia. We suggested glia-induced plasticity of A- and D-current expression as a novel mechanism of long-term modulation of hippocampal neuron excitability.

629.11 Multiple components of IAHP following trains of action potentials in hippocampal sympathetic ganglion neurons. P. Pennefather* and M.V. Sanchez-Vives1, MRC Institute of the City of Hope, Duarte, CA 91010.

We have recently characterized the sequence of events coupling muscarinic receptor activation to the potentiation of the delayed rectifier potassium current (IK) in rat hippocampal CA1 neurons. Ig was recorded from mature rat CA1 neurons in basic slices using the whole-cell patch-clamp method. The currents were recorded during periods of 20 μM carbachol reversibly potentiated Ig (mean=80%) with an onset of action of 1-3 minutes. A reversible decrease in holding current was not associated with changes in the resting conductance was also observed during carbachol application. The intracellular mediators of this enhancement were shown to include a G protein, IP3, DAG, and PKC. This kinase-modulated current was used as an assay to characterize possible actions of ethanol on second messenger systems. Both application of 20 mM ethanol also enhanced Ig with a slower onset of action and recovery rate than carbachol and no associated change in holding current. In addition, incubation of slices with 20 mM ethanol for 10-25 minutes did not consistently inhibit subsequent carbachol enhancement of Ig. Ethanol pretreatment did however reduce the carbachol-induced reduction in holding current.

These preliminary results demonstrate that ethanol, at a clinically relevant concentration, can potentiate Ig in central mammalian neurons. The cellular mechanisms underlying this enhancement are currently under investigation.

Supported by the MRC and an Ontario Graduate Scholarship.

629.12 EFFECTS OF PHOSPHOLIPASE A2-INHIBITORS ON COUPLING OF A2-ADRENERGIC RECEPTORS TO INWARDLY RECTIFYING POTASSIUM CONDUCTANCE IN SUBMUCOSAL NEURONES. R.J. Evans & A. Surprenant*. Vollum Institute, O.H.S.U., Portland, OR 97201

Noradrenaline and somatostatin hyperpolarise enteric submucosal neurons by activating a set of inwardly rectifying potassium channels. Receptor-channel coupling appears to involve only a pertussis toxin-sensitive G-protein because agonists activate the K channels in outside-out membrane patches (Shen et al. 1993). These primary astrocytes were used to study the potential that the production of arachidonic acid and its metabolites may be involved in mediating the response using the PLA2 inhibitors quinacrine and 4-bromophenacyl bromide (4-BPB) and the cyclooxygenase and lipoygenase inhibitor ibuprofen or carbachol (ETYA). Quinacrine (10 μM) reduced the noradrenergic IPSP and hyperpolarisations to the α2 adrenoceptor agonist UK 14304 (100 μM) and somatostatin (10 nM) by 85, 70 and 65% respectively. A similar reduction in the IPSP and the UK 14304 response was found with 4-IBP (10 μM). Quinacrine had no effect on the slow IPSP or the depolarisation in response to substance P, which result from closure of resting and calcium-activated potassium channels, nor on the nicotinic fast EFS. The agonist induced current to UK 14304 and somatostatin shows potential inward rectification at potentials negative to -30 mV; this rectification was reduced by 90% in the presence of quinacrine. ETYA (20 μM) had no effect on the response to UK14304. Our results to date indicate that prostaglandin and eicosanoid metabolites of arachidonic acid are not involved in mediating signal transduction from α2-receptor to potassium channel activation.
629.13 PLUGGING THE LEAK: ADRENERGIC MODULATION OF LEAKAGE POTASSIUM CHANNELS IN RAT THALAMUS.
Peter B. Reiner* and Xue-Ping Wang, Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, BC V6T 1Z3 Canada.

The predominant determinant of the resting potential of excitable cells is a voltage-independent "leakage" potassium current, I_{leak}. Neurortransmitters are capable of inhibiting I_{leak} in mammalian neurons, resulting in a depolarization accompanied by an increase in whole-cell input resistance. Using cell-attached and cell-free patch clamp recordings, we have characterized I_{leak} channels in thalamic neurons studied in slices of rat brain. The channel exhibits a slope conductance of ~24 pS and is highly selective for potassium ions. I_{leak} channels are active at the resting potential and exhibit little voltage-dependence. Alpha_2 adrenergic agonists, known to inhibit macroscopic I_{leak}, reduce the probability of opening of single I_{leak} channels. Modulation of I_{leak} appears to be mediated by a soluble intracellular messenger, as bath application of agonist alters channel kinetics in cell-attached patches. In thalamic neurons, transmitter-induced modulation of I_{leak} channels results in a depolarization which is critically involved in desynchronization of the cortical EEG.

(Supported by MRC)

629.15 β-ADRENERGIC RECEPTOR STIMULATION AND CELL DENSITY REGULATE THE LEVEL OF CONNEXIN43 mRNA IN C6 GLIOMA CELLS.
C. Hsu*, R. L. Margolis and D.-M. Chiang, Biological Psychiatry Branch, National Institute of Mental Health, Bethesda, MD 20892.

The gap junction is a route of rapid intercellular communication among astrocytes and between astrocytes and neurons. The gap junction protein, connexin43, is expressed endogenously in C6 glioma cells. We investigated the regulation of connexin43 mRNA levels in C6 cells in response to a variety of treatments to better understand the factors controlling gap junction expression in astrocytes and neurons. Connexin43 mRNA levels per cell increased with time in culture, reaching levels ~62-fold greater than those expressed on the first day of subculture. 1 µm isoproterenol stimulated a 3 to 4 fold increase in connexin43 mRNA levels within 4 hours in cells of moderate density. β-Adrenergic receptor mRNA was simultaneously down-regulated by this treatment. Somewhat less stimulation by isoproterenol of connexin43 mRNA accumulation was obtained from cells grown at higher cell densities. The increase in connexin43 mRNA induced by isoproterenol could be attenuated 50% or more by the presence of 10 µm colchicine during isoproterenol stimulation, while colchicine alone had little effect. Additional studies using β-adrenergic antagonists and cyclic AMP analogs are underway to determine in more detail the nature of the induction of connexin43 mRNA levels.

629.17 DYNOPHIN REDUCES NMDA-ACTIVATED CURRENTS. L. Chen* and L.-Y. M. Huang*,†, Marine Biomedical Institute* and Department of Physiology and Biophysics†, The University of Texas Medical Branch, Galveston, Texas 77555-0843.

Opioids, such as morphine, has been used widely in the control of pain. Mu-opioid receptors, the preferential binding sites for morphine, clearly play a role in pain modulation. To understand how a κ-opioid receptor participates in pain modulation, we studied the effect of cAMP on Ba and K currents in cultured Drosophila embryonic neurons.

Since these neurons are only 5 µm in diameter, Ca current and modulation mechanisms involving intracellular molecules are likely to washout rapidly when the conventional whole-cell patch clamp technique is used. Therefore, we employed the perforated-patch whole-cell technique, using amphotericin-B to permeabilize the patch. Permeabilization was unreliable in small-opening patch electrodes required for the 5 µm neurons; however, it works much better in large-opening patch electrodes that are used on the 10-15 µm "giant" neurons obtained by arresting cell cleavage with cytochalasin-B.

Our present results suggest that cAMP enhances Ba current but has no effect on K current. With the perforated-patch technique Drosophila Ba current shows no washout. Supported by NSF Grant BNS-8903312.

(Supported by NIH NS11255 and NS10120).

629.18 ELECTRICAL PROPERTIES OF GLUTAMATE-RESISTANT AND GLUTAMATE-SENSITIVE CEREBELLAR GRANULE CELLS. C. Sona*, M.T. Ciotti, D. Mercanti, A. Angelini, P. Calissano, *Institute of Physiology, University of Roma "Tor Vergata", Institute of Neurobiology C.N.R., Roma, Italy.

Cerebellar granule cells in vitro in the presence of a protein complex isolated from rabbit serum (NOAC) develop a phenotype which is in several properties identical to that ensuing in 10% FCS but is markedly different in terms of glutamate sensitivity. NOAC-cultured neurons exhibit a full resistance to the otherwise lethal action of excitatory amino acids (EAAs). This EAA-phenoctype can be induced to become EAA-sensitive (EAA+) when neurons are incubated with another protein complex isolated from rabbit serum. Membrane ionic currents have been recorded in EAA- and EAA+ neurons using the whole cell patch-clamp technique. When K currents were blocked, depolarization commands evoked Na and Ca currents in EAA- and EAA+ neurons. The amplitude of the Na currents was significantly bigger in EAA+ neurons than in EAA- neurons while there was not significantly difference in amplitude in Ca currents in the two groups of neurons. Our preliminary data show that the electrical properties of EAA- and EAA+ neurons are different.
629.19
ZINC POTENTIATES ATP-ACTIVATED INWARD CURRENT IN RAT NODOSE GANGLION NEURONS. Chaying Li*, Robert W. Peoples, Zhiwang Li and Forrest F. Weight. Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The effects of micromolar zinc on membrane inward current activated by extraacellular adenosine 5'-triphosphate (ATP) were studied in freshly isolated adult rat nodose ganglion neurons using the whole-cell patch-clamp technique. In 48 of 50 neurons, zinc, 5 or 10 μM, increased the peak amplitude of current activated by 10 μM ATP by 30% (n=9) and 120% (n=16), respectively, and reduced the rate of decay of the current significantly. In 12 of 50 neurons, zinc decreased the rate of decay of ATP-activated current but had little effect on the peak amplitude of the current. Enhancement of peak amplitude of ATP-activated current by zinc (1-50 μM) was concentration-dependent with an EC50 of 11 μM. In this concentration range zinc did not have any other detectable effect, and did not change the reversal potential of ATP-activated current inward current. The potentiation of membrane conductance was not voltage-dependent between -80 and +60 mV (P>0.25, n=5). Zinc shifted the concentration-response curve for ATP to the left and significantly decreased the EC50 of ATP from 30 to 8 μM. These observations suggest that the modulatory site for zinc action may be located on or near the exterior surface of the ATP receptor-ion channel complex. They also suggest that zinc may enhance the ATP-activated current by increasing the affinity of the receptor for ATP.

629.20
EFFECTS OF CALCIUM AND MAGNESIUM IONS ON SPONTANEOUS OSCILLATION OF MEMBRANE CURRENT IN MAMMALIAN PARA-SYMPATHETIC NEURONS. T. Nishimura and T. Akasu. Department of Physiology, Kurume University School of Medicine, Kurume 830, Japan.

A spontaneous rhythmic outward current (Iso) was recorded from neurons in rabbit vesical paraganglionic adrenal medullary cells voltage clamped in the presence of magnesium and calcium ions. The Iso was abolished by reducing extracellular magnesium ions to less than 1 mM and changing extracellular calcium ions to less than 10 μM. These results suggest that mammalian parasympathetic neurons are similar to sympathetic neurons in terms of their calcium and magnesium sensitivity. In addition, the Iso was not significantly affected by reducing extracellular sodium ions to 20 mM.

629.21
EFFECTS OF INTRACELLULAR SODIUM AND CHLORIDE ACTIVITIES ON THE MEMBRANE PROPERTIES OF CA1 NEURONS. Kevin J. Stanley. Neurology Dept, University of Colorado Health Sciences Center, Denver, CO 80262. 

While cell recordings from different laboratories have reported various conclusions about the effects of intracellular Na and Cl on membrane properties, the results of these studies suggest that the intrinsic activities of Na and Cl may modulate these "membrane properties". The Na and Cl of CA1 neurons were measured in adult hippocampal slices preparations using patch-clamp electrophysiological techniques. All electrode solutions were pH 7.25 and included (in mM) KHEPES 10, MgCl2 2, and variable Na and Cl (tabulated below). K gluconate was added so that the sum of K, Na, Cl, and gluconate was 284 meq L-1. RMPs were corrected for junction potentials. 34C, pH 7.4 artificial cerebrospinal fluid (ACSF) included NaCl, 126; KCl, 25; NaHCO3, 26; CaCl2, 2; MgCl2, 2; NaH2PO4 1.25; and glucose, 10. 800 μM extracellular Na (ECA) was added to the ACSF in some experiments.

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<th>Cl (mM)</th>
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<td>25</td>
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<td>-68</td>
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In the high Cl solutions, ECA both decreased the Rm and diminished a large, slow depolarizing shift of the RMP. Effects of intracellular calcium buffering (10 mM EGTA) were small. These results suggest that electroneutral NaCl cotransport may reduce internal Na in cells recorded with high Cl electrode solutions, thereby decreasing a Na-dependent K conductance which is active at RMP and which significantly affects the Rm.

629.22
COMPUTER SIMULATION OF A CA3 HIPPOCAMPAL NEURON. M. Migliore*, David Jaffe and Daniel Johnstone. Div. of Neuroscience, Baylor College of Medicine, Houston, TX.

Computer simulations of the firing properties of hippocampal CA3 pyramidal neurons were performed using the program NEURON/MODL, which was developed by Mike Hines at Duke University. The model neuron (Jaffe et al., Soc. Neurosci. abstr. 17, 1991) was constructed from a camera lucida drawing of a gold-impregnated CA3 neuron and consisted of 149 compartments, representing the soma and apical and basal dendrites. To study the interplay among synaptic currents during spike-frequency adaptation and bursts, several ionic currents have been included in the model. Based on available voltage- and current-clamp experimental data, we modeled the Ca-independent potassium currents IK, IKCa and IKCa, the Ca-dependent potassium currents IK and IKCa, a fast Na current, INa, and three Ca currents, ICaL, ICaT and ICaD. Calcium buffering, pumping and radial and longitudinal diffusion have also been incorporated into the model. Using a combination of electrophysiological and fluorescence imaging results, distributions of several of these channels were nonuniformly placed in different regions of the dendrites. The results from the simulations can qualitatively reproduce a number of the repetitive firing characteristics of these pyramidal neurons under different conditions of injected current and pharmacological manipulations. (Supported by NIH grants MH44754 and 48431 and the Keck Foundation.)

629.23

The effect of axotomy on the electrical properties of X-organ neurons of the crayfish was investigated with microelectrode techniques. These neurons are important effectors of the calcium-extrusion system which generate the Iso in rabbit VPG neurons. Apartado Postal 14-740, Mexico, D.F. 07000. MEXICO.

The calcium-extrusion system which generates the Iso in rabbit VPG neurons was investigated. The Iso occurs under a variety of conditions, including stimulation of the cell body, change in extracellular calcium concentration, and change in external magnesium ions (6 mM). Nifedipine (10 μM), verapamil (1 μM) and ω-conotoxin (1 μM) did not alter the Iso. Application of BAPTA-AM (300 μM, 30 min), a calcium-chelator acting in cytosol, eliminated the Iso. Subsequent calcium-loading into cells restored the Iso. The axotomy of crayfish calceus (CICR) produces a large depolarizing shift of the RMP to -80 mV (P > 0.25, n = 5). Zinc shifted the concentration-response curve for ATP to the left and significantly decreased the EC50 of ATP from 30 to 8 μM. These observations suggest that the modulatory site for zinc action may be located on or near the exterior surface of the ATP receptor-ion channel complex. They also suggest that zinc may enhance the ATP-activated current by increasing the affinity of the receptor for ATP.

629.24

In a behavioral screen of 2,000 enhancer trap lines we isolated one (designated 2206) in which the P-element had inserted into region 9SB of the salivary gland chromosomes, a site of the previously cloned cDNA for the α-subunit of the Drosophila Na+,K+-ATPase. The following evidence indicates that 2206 is a hypomorphic mutation resulting from the insertion of the P-element into the regulatory region of the gene: (1) 2206 homozygous flies were only about 35% of the wild type size. (4) A monoclonal antibody to the α-subunit stains much less intensely in certain tissues of 2206 than it does in control flies. (2) They are bang-sensitive, undergoing brief paralysis in response to mechanical agitation. This behavior is mimicked by injection of ouabain into wild type flies. (3) Excision of the transposon leads to a reversion of the bang-sensitive phenotype and restores wild type resistance to ouabain. (4) A monoclonal antibody to the α-subunit stains much less intensely in certain tissues of 2206 than it does in control flies. Quantitative protein immunoblots show that in the mutants only about 35% of the wild type quantity of pump protein is made. (5) In Northern blots the cDNA to the regulatory region including the previously reported mRNAs but shows quantitatively reduced hybridization to the 2206 cDNA. (6) The 2206 cDNA is a guinea pig muscle cDNA clone. In Northern blots the cDNA to the regulatory region including the previously reported mRNAs but shows quantitatively reduced hybridization to the 2206 cDNA. (7) The 2206 cDNA is a guinea pig muscle cDNA clone.
ACETYLCHOLINE: CHOLINE ACETYLTRANSFERASE AND CHOLINESTERASE

630.1

Choline acetyltransferase (ChaT, E.C. 2.3.1.6) catalyzes the biosynthesis of the neurotransmitter acetylcholine using choline and acetyl-CoA and serves as the most specific marker yet known for cholinergic neurons. The detailed characterization of this enzyme at the molecular level has been hampered by its low abundance from natural sources. Previous attempts to express recombinant Drosophila or rat ChaT in E. coli resulted in high levels of inactive insoluble enzyme and only modest levels of soluble active enzyme. We report here the optimization of expression of rat ChaT in its active form in E. coli, yielding approximately 50 mg of enzyme per liter of culture (estimated to be equivalent to the amount of enzyme contained in 20,000 rat brains). A facile purification scheme was developed for the recombinant enzyme using a poly-histidine affinity tag which permits homogeneous enzyme to be obtained in a single day. Finally, the crystallization of the recombinant enzyme has been achieved which should pave the way for the determination of the enzyme structure.

630.2
FEEDBACK REGULATION OF CHOLINE ACETYLTRANSFERASE EXPRESSION IN DROSOPHILA. V. Andreani, T. Kitamoto and P.M. Salvatores. *Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

We have examined the possibility that transcriptional regulation plays an important part in feedback regulation of the Drosophila choline acetyltransferase (ChaT) gene. We constructed transgenic flies carrying a lacZ reporter gene whose expression was directed by the 5' flanking DNA of the ChaT gene. The fusion gene was introduced into Drosophila with either a wild type or temperature sensitive Cha genetic background. Compared to wild type flies, temperature sensitive Cha mutants show lower ChaT activity and higher ChaT mRNA levels. At a restrictive temperature, ChaT activity and ChaT mRNA levels decrease in mutants but increase in wild-type flies. If the 5' flanking DNA directing reporter gene expression contains elements responsible for this feedback regulation, the levels of mRNA coding for reporter gene should change in parallel with those of ChaT mRNA. It will also be possible to map the elements responsible for feedback regulation by using different amount of 5' flanking DNA. We are now analyzing the reporter gene expression directed by 7.4 kb of 5' flanking DNA. This DNA has been shown to contain most, if not all, of the cis elements required for correct temporal and spatial expression of ChaT. Our preliminary results indicate that higher β-galactosidase activity is present in temperature sensitive Cha mutants relative to wild type at a permissive temperature. The β-galactosidase activity also decreases in flies at a restrictive temperature. These results may suggest that 7.4 kb of 5' flanking DNA is involved in feedback regulation of ChaT.

630.3

We have analyzed the distribution of putative cholinergic neurons in whole mount preparations of Drosophila melanogaster. Cholinergic neurons were visualized by X-gal staining of P-element transformed flies constructed with a fusion gene containing bacterial LacZ. LacZ expression was controlled by various amounts of the 5' flanking DNA of the Drosophila choline acetyltransferase gene. We have previously demonstrated that cryostat sections of transgenic flies containing 7.4 kb of 5' flanking DNA express LacZ in a detailed pattern similar to the known distribution of ChaT protein. Whole mount staining of these same flies should thus represent the overall distribution of cholinergic neurons in the fly. X-gal staining could be observed in most, but not all, of the areas of the CNS and PNS. Removal of the distal part of the 5' flanking DNA resulted in a dramatic reduction of X-gal staining in the CNS. Therefore, both the 7.4 kb DNA directed strong lacZ expression in leg sensory neurons but the 1.2 kb DNA did not. Our results suggest that ChaT expression is regulated differentially in the CNS and PNS.

630.4
EXPRESSION AND LOCALIZATION OF CHOLINE ACETYLTRANSFERASE AND THE unc-17 GENE PRODUCT IN THE NEMATODE, C. elegans. J. B. Rand*. N. P. Han and J. B. Rand. Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

In C. elegans, cha-1, the gene that encodes choline acetyltransferase (ChaT), is part of a complex genetic locus with unc-17. The function of the unc-17 gene product is unknown, but defects in this gene, like defects in cha-1, cause uncordinated locomotion and resistance to cholinesterase inhibitors. To study the function and distribution of these two gene products, we are expressing them as fusion proteins and generating specific antibodies for immunocytochemical studies.

The full length cDNA sequences from the cha-1 and unc-17 genes have been cloned into pMAL plasmid expression vectors to produce fusion proteins in E. coli. The fusion proteins were induced, purified, and cleaved to yield ChaT or unc-17 protein; these cleaved proteins were used as immunogens. Peptides designed from the known cDNA sequences were also used as immunogens. Polyclonal sera were produced in rabbits and chickens; a subset of these sera specifically recognized the ChaT or unc-17 fusion protein on Western blots. However, the sera exhibited high nonspecific staining of C. elegans. Therefore, they have been affinity purified with the fusion proteins before use in immunocytochemistry. The affinity-purified sera are being used to identify the ChaT and unc-17 containing neurons in the ventral nerve cord and ganglia, as well as to identify staining in any non-neuronal cells. This identification will be simplified by the fact that the identity and origin of all of the cells in C. elegans have been described. Supported by grants from NSF and NIGMS.

630.5

cha-1 and unc-17 are parts of a complex locus and transcription unit. cha-1 encodes choline acetyltransferase, and unc-17 encodes a unique 58 kDa protein, uncharacterized function, believed to be involved in acetylcholine metabolism or release. unc-17 is nested within a long cha-1 intron, and the two transcripts share some 5' sequences. The entire genomic region (11.5 kb) has been sequenced. We screened 3 cDNA libraries, and isolated 3 independent cha-1 cDNAs and 4 independent unc-17 cDNAs. Although cha-1 and unc-17 contain no coding sequence in common, all of the cDNAs share a 60 bp 5'-untranslated exon. The 2 cDNAs extending furthest in the 5' direction (1 cha-1 and 1 unc-17) also contain sequences apparently derived from the trans-spliced leader SL1. Primer extension analysis revealed heterogeneity within each transcript class. A cha-1 specific primer yields a minor product which is the predicted length for a trans-spliced RNA, and a major product of similar length. The unc-17 specific primer gives rise to several extension products: the most abundant one is the predicted size for trans-spliced RNA, and a minor product corresponds to the length of the major cha-1 extension product. Supported by a grant from NIGMS.

Several cholinesterase inhibitors (ChEI) are presently being clinically evaluated for enhancement of cholinergic function in Alzheimer's disease (AD) patients. We postulated that the ability of ChEI to ameliorate the cholinergic deficit in AD is related to their ability to maintain long-lasting, non-toxic steady state levels of ACh in cortex (Boeker and Giaconia, 1988). We have modified the HPLC-EC method for acetylcholine (ACh) to detect femtomole levels of ACh in microdialysis fluid from rat frontal cortex without using a ChEI in the probe to elevate ACh levels. Using this methodology we have compared the effects of two first generation ChEI, pyridostigmine (PY) and tetrahydroaminoacridine (THA) and two second generation ChEI, heptylpyridostigmine (HEP) and MDL-73,745, in increasing and maintaining cortical extracellular ACh levels in the rat. Disimilar magnitudes of ChEI activity were seen. Although all four ChEI are capable to significantly raise cortical ACh levels, the effects of HEP and MDL 73,745 on ACh are more long-lasting and are associated to less cholinergic side effects than PY and THA at doses producing comparable AChE inhibition. It appears that ChE activity inhibition is not the sole determinant of extracellular ACh levels and cholinergic side effects. Pyridostigmine or HEP elicited the same maximal effect on ACh levels in the diastase, however, ChE inhibition differed markedly between the two. This may reflect differences in the pharmacological profiles of these ChEI. Therefore, the use of ChEI inhibitors, currently used is not sufficient to predict effects on extracellular cortical ACh levels. Additional factors may influence this relationship and identification of these factors may improve prediction of therapeutic efficacy of ChEI treatment in AD. (Supported in part by NIA Core Grant #P30 AG068014). REFERENCE: Becker, R. and E. Giaconia, Mechanisms of cholinesterase inhibition in senile dementia of the Alzheimer type: clinical, pharmacological and therapeutic aspects. Drug Dev. Res. 12:163-195, 1988.

360.8  STANDARDS FOR ACETYLCHOLINESTERASE HISTOCHEMISTRY PERMIT QUANTIFICATION OF ENZYME IN TISSUE SECTIONS USING COMPUTER AIDED DENSITOMETRY. B. W. Foster*, M. B. Moore, D. L. Rosen. Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118.

Acetylcholinesterase (AChE) histochemistry is an important method for determining the distribution of cholinergic nerve terminals, but is impractical for quantification of AChE in regions unavailable for dissection and biochemical analysis, such as the subfields of the hippocampus. We have developed a series of AChE standards that can be verified biocemically and used to calibrate the optical density of AChE reaction product in tissue sections. Membrane bound AChE (EC 3.1.1.7 Sigma) was extracted using Triton X100 and solubilized in a gelatin solution which was then frozen in isopentane, cut on a cryostat at 15 μm and processed histochemically by the method of Tago along with 15 μm fresh frozen brain sections. The concentration of AChE in the standards was determined by ¹⁴C-acetylcholine degradation (measured as μmol ACh degraded/min/gram at 37°C and pH 8). The variability of optical density in different slides of the standards was less than 7%, indicating uniform distribution of the enzyme. The relationship of optical density to the biochemically determined AChE concentrations in the standards was best fit by a linear model (p<0.01). Optical density of standards is also linearly related (p<0.05) to duration of histochemical incubation. Measurements using standards to calibrate optical density. AChE concentration was determined in 25 hippocampal subfields of normal adult rats. Highest concentrations of AChE were found in the piriform layers of Ammon's horn (13 to 11 units of AChE) and the infragranular region of the dentate gyrus (11 units), while stratum radiatum contained the lowest concentrations (3.7 to 5.2 units). The ability to detect subtle quantitative changes can help refine knowledge of the hippocampus, which may have several smaller routes of cholinergic innervation other than the fornix. "Supported by NIH training grant NS07152, research grants AG00001, NS16841, AG04321, and Alzheimer's Association Grant RG-89-116."

360.9  DISTRIBUTION OF BUTYRYLCHOLINESTERASE IN THE RAT BRAIN. S. Darveyh, A.J. Smereczynsky and D.A. Hopkins*. Department of Anatomy and Neurobiology, Dabholive University, Halfax, N.S. Canada E1K 4H7.

Butyrylcholinesterase (BuChE), EC 3.1.1.8, has a widespread distribution not only in brain blood vessels and glia but also in neurons and neuropil of selected regions of the central nervous system (Friede, 1967). The function of this enzyme in neurotransmission is unknown but BuChE has been implicated in neurodevelopment, neuropathology and drug metabolism. The distribution of BuChE in the rat was mapped following a standard biochemical method and compared with the distribution of cholinergic neurons. BuChE is co-localized with some but not all cholinergic neurons and is found in distinct populations of non-cholinergic neurons. Similar relationships were seen for neuropil staining. In the medulla oblongata, BuChE stained was prominent in some but not all motoneurons, the subnucleus interpolaris of spinal v, medial reticular formation and parts of the vestibular complex. In the pons, BuChE was concentrated in the caudal cholinergic cell groups and lateral reticular formation. In the mesencephalon, the enzyme was present in parasympathetic motoneurons and the neuropil of the interpeduncular nucleus and superior colliculus. Several thalamic nuclei were heavily stained as were parts of the basal forebrain. Sporadic neurons of the neocortex were intensely stained as was the neuropil of the cingulate cortex. A detailed map of BuChE distribution in the rat brain will provide a guide for investigations into possible neural functions of this enzyme. Supported by MRC (MT-7369) and the Scottish Rite Charitable Foundation of Canada.

360.10  REACTIVATION OF TABUN-INHIBITED AChE BY BISQUATERNARY OXIMES, RELATED TO PYRIDOSTIGMINE PRETREATMENT. G. Antaki, T. Babintovitz, G. Cohen, G. Zembor, R. Adami and E. Hamev, TIBS F.O.Box 19, 70450 Ness Ziona, Israel.

Certain oximes such as toxogonin and HI-6 together with anticholinergics such as physostigmine and neostigmine cause poisoning by organophosphates. Pretreatment with pyridostigmine (PY) increases their protection ratio (PR). We have studied the reactivation of tabun-inhibited PBS-AChE elicited by toxogonin, HI-7, AB-13, HI-6 and AB-8. The bioinorganic rate constants for reactivation obtained for toxogonin, HI-7 and AB-13 were 157, 18.7 and 12.5 M⁻¹min⁻¹, respectively, whereas AB-8 and HI-6 showed less reactivation potencies. The reactivation data correlated well with the PR values obtained in conjunction with atropine and benactyzine in mice and guinea pigs following poisoning. In mice, HI-6 and toxogonin were added as a pretreatment the PR values markedly increased (e.g. in guinea pigs, HI-6 from 3.8 to 50 and AB-8 from 3.3 to 45). Pretreatment with HI-7 could not be correlated to the reactivation data. Reactivation of PY-inhibited PBS-AChE displayed similar kinetics for HI-6, HI-7 and toxogonin (τ₁/₂=15-30 min) whereas the rate obtained for AB-8 and AB-13 was equal to spontaneous reactivation (t₅₀=50 min). Thus, it seems that the anticholinergic agent atabumin with PY pretreatment can be related only to the reactivation rate of carbamoyl-AChE. It is conceivable that different conformational changes occur upon formation of ternary binding complexes comprised of PY-AChE-oxime.


Norpyridostigmin, is a new centrally active, reversible acetylcholinesterase (AChE) inhibitor. Norpyridostigmin is a derivative of pyridostigmin, the latter does not cross the blood-brain barrier. We assessed the effectiveness of Norpyridostigmin, on the level of acetylcholine (ACh) and the activity of AChE in rat brain. ACh and AChE were measured following an injection (i.p.) of Norpyridostigmin tosylate (30mg/kg). Control rats received a saline injection. ACh levels were measured five times intervals (15-180 min) following injection. Rats were killed with a focused microwave apparatus, to instantaneously denature AChE. A HPLC-EC technique, with an immobilized cholinesterase (Bioanalytical Systems, Inc.), was used to measure the level of ACh. AChE activity was measured across four time intervals (15-120 min), using a radioassay enzyme. ACh was increased by almost 50% at 20 min, and was still above control at 3 hr. AChE activity was decreased by almost 70% at 30 min, and was still inhibited at 71% at 120 min. Norpyridostigmin markedly inhibits the breakdown of ACh by AChE for several hours. Behavioral studies done in our laboratory, demonstrated that Norpyridostigmin attenuated the learning and memory deficit observed in the rodent basalis magnocellularis in the rat. In Alzheimer's disease (AD), a profound cholinergic deficit exists, which has been linked to the memory impairment associated with the disease. Our data suggests that Norpyridostigmin might be useful in the treatment of AD.

The biophysical properties of the synaptic activation of AChRs in chick lumbar sympathetic neurons, and the developmental regulation of nAChR subunit expression and function in chick ganglia, were assessed. In vitro nicotinic responses were compared with those from cultures grown on collagen-coated substrate in the absence of muscle or muscle cell membranes. During culture, the responsiveness to ACh was first decreased, and then increased, with a return to baseline levels by 3-4 days postplating. Forskolin (1 μM) and IBMX (1 mM), soon after plating, had a variable effect on current amplitude in others. However, by 3 to 4 days postplating forskolin and IBMX have no effect on the ACh-induced currents in neurons grown in culture. Exposure of neurons to muscle membranes for 24 hours increased ACh-induced currents from 128 ±45 nA (normal target of these neurons, restores ACh responsiveness. The 49 kD protein was identified as the α5 gene product assembles with multiple subunit-specific monoclonal antibodies indicated that in chick ganglia much of the α5 gene product is co-assembled with α3 and α4 subunits together, but not with α6 subunits. In brain, the α5 gene product was found associated with α7 and α6 subunits, with a3 subunits, and, to a small extent, with both together. No α5 was found associated with α7 or α8 subunits. Other AChR gene products have yet to be tested for co-assembled with α5. The results show that neuronal AChRs can have as many as three kinds of subunits with at least two being of the α-type. (NS 12601 & 25916)


The developmental changes in nAChR subunit expression in chick ganglia were assessed. Chicks were grown on collagen-coated substrate in the absence of muscle or muscle cell membranes. At this time, forskolin and IBMX had no effect on the ACh-induced currents in neurons grown in culture. Exposure of neurons to muscle membranes for 24 hours increased ACh-induced currents from 128 ±45 nA (normal target of these neurons, restores ACh responsiveness. The 49 kD protein was identified as the α5 gene product assembles with multiple subunit-specific monoclonal antibodies indicated that in chick ganglia much of the α5 gene product is co-assembled with α3 and α4 subunits together, but not with α6 subunits. In brain, the α5 gene product was found associated with α7 and α6 subunits, with a3 subunits, and, to a small extent, with both together. No α5 was found associated with α7 or α8 subunits. Other AChR gene products have yet to be tested for co-assembled with α5. The results show that neuronal AChRs can have as many as three kinds of subunits with at least two being of the α-type. (NS 12601 & 25916)

Innervation of Xenopus nicotinic acetylcholine receptor (nAChR) protein and mRNA levels in muscle. In developing motoneurons, innervation as well as intrinsic factors and retrograde signals from the target tissue may be important for vulnerability to thyroid hormone. To establish the role of cell-to-cell interactions in inducing nAChR expression in chick ciliary ganglion motoneurons in situ, we have surgically removed the preganglionic nerve (at ED 3.5-4) or the target tissue (the eye, at ED 2) prior to synapse formation. Ganglia were examined at ED 8. Previous studies have demonstrated that ciliary ganglion neurons develop normally in the absence of targets or target tissue interactions up to ED 9.

α7 subunit was subcloned into RT-PCR and mutated internal standards. In input-deprived ganglia, both α3 and β4 mRNA levels are reduced 30% as compared to control ganglion values at ED 8. Target tissue-deprived ganglia have 20% lower α3 and β4 mRNA levels relative to control ganglia. In comparison, ganglia deprived of both source of inputs and the target tissue have even greater reductions in α3 and β4 mRNA levels, exhibiting 80% declines relative to controls. Qualitatively similar changes were observed in internal nAChR levels by labeling frozen sections of operated and control ganglia with an anti-AChR mAb. The results demonstrate that both presynaptic input and retrograde signals from the target tissue regulate nAChR protein and mRNA levels in developing neurons. Supported by NIH NS 21725, the Pfeiffer Fond. and MDA.

The rat α7 subunit encodes a nicotinic ion channel highly permeable to calcium. P. Seguela, W. Wadiche, K. Miller, A.C.S. Costa*, L. A. Danil and I. W. Patrick. Division of Neuroscience, Department of Neurological Surgery/Physiology & Biophysics, Baylor College of Medicine, Houston, TX 77030.

We isolated a full-length clone coding for the rat neuronal nicotinic receptor α7 subunit and tested its pharmacological and functional properties in the Xenopus oocyte expression system. Homomeric α7 nicotinic receptors displayed a characteristic profile of sensitivity to agonists (nicotinic > dMPF > acetylcholine) and antagonists (α-BTX > D-tubocurarine > strychnine). Permeability of α7 channels to Ca2+ ions was suggested by a dramatic decrease of nicotine-induced current after specific blockade of the endogenous Ca2+-activated chloride channels with niflumic acid and flufenamic acid. In the presence of high external Cl-, application of agonist produces an inward current followed by an outward current sensitive to Cl- channels blockers. We compared the reversal potential shift of the ligand-induced current in 1mM vs 10 mM external Ca2+ under conditions where Cl- currents are minimal. We measured a shift of 10 ± 0.5 mV, larger than previously reported for muscle and neuronal nicotinic receptors. We conclude that the α7 receptors are highly permeable to Ca2+, with an apparent rP channel in the case of the non-MDsubtype of glutamate receptor. The functional properties and the expression pattern of this subunit in the rat brain suggest that α7-containing acetylcholine-gated channels play a significant role in triggering post-synaptic Ca2+-mediated events in the limbic system in general and in the hippocampus in particular. Supported by NINDS, NIDA, NIH and DOD.


Muscle acetylcholine receptor (AChR) synthesis is closely regulated by the state of neuromuscular activity. Denervation causes an increase in AChR subunit gene expression, while direct electrical stimulation of muscle down regulates AChR subunits. We have previously shown that treatment of cultured (a cholinergic agonist) decreases AChR β-subunit message levels in primary rat muscle cultures at 24 hours post-treatment. However, the early genomic regulatory events induced by cyclophilin treatment are not known. Immediate early genes (IEGs), that encode transcription factors and are induced rapidly after cell surface stimulation, are candidates for such a role. We studied the expression of 4 IEGs (zif268, c-jun, nur77 and junB) in the mouse skeletal muscle cell line - C2 - which normally fuses and expresses AChRs. Treatment of C2 mouse skeletal muscle cells with carbobioch induced increases zif268, c-jun and nur77 mRNA levels, while junB message levels showed no consistent change. The mRNA levels were increased at 1 hour, peaked at 3 hours, and were back to basal levels by 6 hours. This effect was blocked by cyclophilin pre-treatment. In addition, A23187 and Veratridine, which cause an influx of Ca2+ and Na+ ions respectively, induced a similar pattern of IEG expression. This suggests a role for IEG expression in regulating skeletal muscle properties in response to neuromuscular activity.


The function of the neuronal nicotinic α2b-BGT receptor is currently unclear although the α2 subunit has been implicated in growth related activities. Presently, 10-6 and 10-7 M decrease nerve growth factor induced neuritic outgrowth in PC12 cells. This effect was observed as early as 1 day after exposure to the agonist and persisted for at least 7 days. Interestingly, α-BGT, at 3x10-6 M and 10-8 M, did not decrease the decrease in neurite formation that occurred after nicotine exposure. These concentrations of the α2-BGT correalted very well with those which resulted in a block of [3H]-BGT binding to PC12 cells. Thymopoietin (10-7 M), a thymic polypeptide, which interacts potently and specifically with the α2 subunit, also prevented the nicotine induced decrease in process formation; again the concentration required to affect this was the same. Interestingly, α-BGT, that required to inhibit [3H]-BGT binding. Cell numbers were not altered after exposure to any of these agents, thus alterations in neurite outgrowth were not due to changes in cell proliferation. The effect of thymopoietin was also tested on nicotine receptor mediated neurite outgrowth; no change was observed indicating that thymopoietin specifically affects the nicotinic α2BGT receptor population. The present results suggest a functional role for the neuronal nicotinic α-BGT receptor in regulating neurite outgrowth and for thymopoietin as an endogenous ligand with such a role at the α2-BGT site. Supported by the MRC (Canada).

A vertebrate α-bungarotoxin (α-Bgt)-sensitive nicotinic acetylcholine receptor (nAChR) subunit, α7, has been cloned and forms homo-oligomeric channels when expressed in Xenopus oocytes. Expression of an analogous receptor subunit from a locust, ARL2, also results in functional homomeric channels sensitive to α-Bgt2. We have compared the properties of these two putative nAChR using cDNA expression in oocytes. In both cases, agonist-evoked current (IV = -70 mV) for (nM) α-Bgt and cystine modulated sigmoidal relationships between current amplitude and agonist concentration. α7 was much more sensitive to α-Bgt than ARL2. Strong inward rectification of the current-voltage relationship was evident in both cases. Whereas α7 channels activate and desensitize rapidly over approx 2s, ARL2 activates slowly and shows no desensitization in the presence of agonist for 4s. Both types of channel are irreversibly blocked by α-cobra toxin and reversibly by methyllycaconitine. These expressed subunits allow direct comparison of the specific, activating pharmacophore of each receptor, using families of specific ligands including cystine, anatoxin and piperazone-based compounds.

2. Marshall et al. (1990) EMBO J, 9, 4891-4898. Acknowledgments to RJR Tobacco Co. & Shell Research Ltd. for support, and M. Ballivet for α7 cDNA.

631.14 EXTERNAL Ca2+ POTENTIALLY ENHANCES NEURONAL NICOTINIC ACETYLChOLINE RECEPTOR CURRENTS. Mariano Amador and John A. Dani*. Division of Neurosci., and Dept. of Molec. Phys. & Biop., Baylor College of Medicine, Houston, TX 77030.

Recently, we found that some subtypes of neuronal nicotinic acetylcholine receptors (nAChRs) are highly permeable to Ca2+ and are modulated by external Ca2+ (Verney et al., 1992 Neuron 8:127). As the external concentration of Ca2+ is increased, whole-cell currents through muscle nAChRs decrease but currents through neuronal nAChRs increase. As the external Ca2+ is decreased, whole-cell neuronal nAChR currents decrease, but small currents are still seen in Ca2+-free solutions.

Patch-clamp studies of chromaffin cells indicated that Ca2+ acts externally on the nAChRs rather than altering an intracellular enzyme cascade. Ca2+ enhancement of NnAChR currents is very rapid, is reversible and repeatable in cells internally perfused with 20 mM BAPTA, does not require exogenous enzymes or a source of phosphate, is not voltage dependent, and is seen in cell-free patches of membrane. In addition, the amplitude of the single-channel currents decreases (not increases) as external Ca2+ is increased. These results suggest that an influx of Ca2+ is not required to observe the enhancement of agonist-induced NnAChR responses.

Activity-dependent fluctuations in extracellular Ca2+ have been reported throughout the central nervous system, and the Ca2+-dependent modulation of NnAChRs occurs over a range that includes physiological levels of Ca2+. Therefore, activity-dependent changes in the concentration of external Ca2+ could alter NnAChR responses and thereby influence the efficacy of neuronal nicotinic synapses.


To identify regions of the β subunit that influence the pharmacological selectivity of neuronal nicotinic receptors we constructed chimeras of the β2 and β4 subunits and expressed them in Xenopus oocytes. Peak macroscopic currents elicited by 30 μM acetylcholine (ACh), cytisine (CYT), nicotine (NIC), and 100 μM tetraethylammonium (TEA) were measured using two-electrode voltage clamp. For the β2 wild-type receptor, the relative amplitudes of the CYT, NIC, and TMA responses compared to ACh were 0.02 ± 0.01 (mean ± sd, n = 10), 0.3 ± 0.2 (n = 3), and 0.6 ± 0.5 (n = 3). For the β2 wild-type, the relative responses were 2.6 ± 0.2 (n = 10), 1.3 ± 0.2 (n = 3), and 1.8 ± 0.5 (n = 3). The external regions of β2 and β4 (N-terminal to M1) account for almost all of the effect of the β subunits on selectivity. For TMA, chimeras with the first 109 or fewer amino acids from β4 and the remainder from β2 showed β2-like sensitivity. Chimeras with the first 111 or more amino acids from β4 showed β4-like sensitivity. For CYT, chimeras with the first 92 or fewer amino acids from β4 showed β2-like sensitivity, although there was an increase in the relative response for chimeras with ≥ the first 20 residues of β4. Chimeras with the first 109 or more amino acids from β4 behaved as the β4 wild-type. There was no distinct region that accounted for NIC sensitivity. The results show that the 109-111 region accounts for most of the difference in TMA sensitivity, the 92-109 region accounts for most of the difference in CYT sensitivity, and that no single region of the external portion of the β subunit accounts for the difference in NIC sensitivity. (Support: NS-11756, CA. TRDRP, MDA)

631.17 GENE TRANSSCRIPTS FOR THE nAChR SUBUNIT, B4, ARE DISTRIBUTED IN MULTIPLE AREAS OF THE RAT CNS. E. Dinsley-Miller, S. B. Sand*, and J. Patrick. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Previous in situ hybridization experiments found that beta 4 (β4) neuronal nicotinic acetylcholine receptor (nAChR) transcripts were found only in the medial habenula (MBH). Co-expression in Xenopus oocytes of the β4 subunit and any one of three ligand-binding or alpha subunits results in the formation of functional nAChR. Comparisons between the pharmacology of nAChR expressed in oocytes and the pharmacology of nAChR’s found in the rat CNS prompted a further investigation of the localization of transcripts encoding the β4 nAChR subunit. Using two β4-specific cRNA probes, in situ hybridization was performed in rat brain. β4 mRNA was detected at high levels in the presubiculum, parasubiculum, subiculum and dentate gyrus of the hippocampal formation, in layer IV of the isocortex, in the medial habenula, in the interpeduncular nucleus, and in the oculosaccular and trigeminal motor nerve nuclei. Moderate hybridization signals were seen in the isocortex (layers I-III), in olfactory regions, in fields CA1 through CA4 of Ammon’s horn and the entorhinal cortex of the hippocampal formation, in the supramammillary nucleus, in the pontine nucleus, in the cerebellum, and in the locus coeruleus. No signal was detected in the septum, basal ganglia, sensory portions of the brainstem, or spinal cord.

631.18 SUBUNIT SPECIFIC ANTAGONISTS PREPARED AGAINST NEURONAL nAChR’s. K. Dinsley-Miller, S. E. Neff, D. Chal, and J. Patrick*. Division of Neuroscience, Baylor College of Medicine, Houston TX 77030.

The nicotinic acetylcholine receptor (nAChR) gene family includes, to date, ten genes whose transcripts are found in a variety of central and peripheral nervous system structures. Molecular cloning has allowed the expression and study of these receptors in vitro but elucidation of receptor composition and localization in rat brain has been hampered by a lack of biochemical probes specific for the extracellular domain of the native form of each subunit. Our strategies for preparing functional antibodies selective for seven of these highly related proteins consisted of 1) immunizing rabbits with bacterially expressed recombinant protein representing the N-terminal extracellular region of each nAChR subunit; 2) affinity purification of antibodies against a synthetic peptide prepared for each subunit in the region corresponding to amino acid residues 69-83 of the α11 subunit of the muscle nAChR (this portion of the α1 subunit is within the main immunogenic region recognized by antibodies of most tobacco-sensitive patients); 3) subtracting cross-reactive antibodies by adsorbing against recombinant proteins of homologous subunits. Subunit specificity of antisera have been evaluated by immunoblot of recombinant protein, immunohistochemistry, and affinity purification of receptor subunits from expressed in Xenopus oocytes rat brain.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
**Excitatory Amino Acids: Receptors VIII**

**632.1** Calcium dependent desensitization of hippocampal NMDA receptors. G. Lonart* and K.M. Johnson. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555-1031.

Prolonged stimulation of NMDA receptors can lead to desensitization. In this study, NMDA-stimulated fractional release of [3H]-norepinephrine ([3H]-NE) from cross-chopped rat hippocampal slices was utilized as a functional measure of NMDA receptor activity. In the second 5 min fraction of continuous superfusion with 100 μM NMDA, [3H]-NE release reached a maximum level (decreased from baseline) and then rapidly declined. The response to 3 μM onomonoxy, a calcium ionophore, did not decline during the same time course. Introduction of additional NMDA (300 μM, 30 sec pulse) at the 30th min of superfusion with 100 μM NMDA had no significant effect. However, addition of 300 μM kainate under the same conditions produced a significant increase in the [3H]-NE release. These observations indicate that a homologous desensitization and that a non-specific change in the substrates underlying vescular release does not account for the decay in the NMDA response. Under conditions of reduced extracellular Ca2+, the response to 100 μM NMDA declined with a slower rate, and subsequent stimulation with 300 μM NMDA produced a significant increase in the [3H]-NE release. This suggests that the NMDA receptors are desensitized by a mechanism which is dependent upon extracellular Ca2+ concentration. Ongoing experiments are being carried out to test possible involvement of protein kinases and phosphatases in this paradigm. Supported by DA-02073.

**632.2** The role of excitatory amino acid receptors and calcium pools in the regulation of nitric oxide synthase (NOS) in cortical slices. S. Alagarsamy*, G. Lonart and K.M. Johnson. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77555-1031.

Nitric oxide (NO) is a putative, calcium-dependent, diffusible second messenger, synthesized from L-arginine (an irreversible inhibitor of NOS). Raising intracellular calcium levels by addition of 100 μM caffeine, a calcium ionophore, did not affect the response to NMDA. The effects of calcium pools on the production of NO from cross-chopped rat hippocampal slices were assessed by utilizing [3H]-nitro-arginine (an irreversible inhibitor of NOS). The results indicate that calcium pools regulate NOS activity. Supported by USPHS NS23807.


In cerebellar slices from adult male Sprague-Dawley rats, kainate, AMPA, or NMDA increased cGMP levels. Noncompetitive NMDA receptor antagonists (MK-801), glycine antagonists (7-chloro-kynurenate), and competitive NMDA antagonists (CPP) inhibited the NMDA-stimulated cGMP. At concentrations that inhibit NMDA-stimulated cGMP, NMDA receptor antagonists do not inhibit the increase in cGMP with kainate. The AMPA receptor antagonist NBQX inhibited kainate-stimulated cGMP but not NMDA-stimulated cGMP. The response to kainate is larger than that seen with AMPA, and AMPA inhibits the response to kainate, consistent with mediation through a single receptor. Treatment with this lectin concanavalin A can increase the magnitude of cGMP increases with AMPA, while not increasing the responses to kainate. Antagonists to voltage sensitive calcium channels inhibit the increase in cGMP with NMDA, but do not inhibit the binding of the noncompetitive NMDA antagonist [3H]-TCP. The results demonstrate excitatory amino acid receptors regulating guanylate cyclase in a calcium dependent manner. Kainate and AMPA seem to interact with a single receptor in this preparation.


β-oxalylamino-L-alanine (BOAA) causes lathyrism, a human disease of upper motor neurons. BOAA is thought to be an excitatory amino acid agonist, and may act at the AMPA receptor. We have characterized long term potentiation (LTP) in rat motor cortex slices and evaluated the effects of bathing perfusion of BOAA on the magnitude of the population evoked response on white matter stimulation, and on the induction and expression of LTP. Since NMDA receptors are required for LTP induction and AMPA receptors for LTP expression, our hypothesis is that if BOAA activates and desensitizes AMPA receptors on upper motor neurons, there should be a reduction in LTP expression without effect on induction. The peak of the population evoked response in motor cortex was reversibly reduced by 50% upon perfusion of BOAA at 10-6 M. Tetanic stimulation (TS 100 Hz, 2s) induced LTP of the population evoked response in layer (L) II. This potentiation persisted for up to 5 hours and its induction was blocked by 10-5 M AP-5, when applied before TS. In L V however, the same TS induced LTP only when the normal Ringer solution was replaced with a solution containing low Mg2+ (0.5 mM), which should increase currents through NMDA channels. This may be due to the difference in the number of NMDA receptors within the cortical layers. The density of NMDA receptors is high in L II, whereas kainate receptors are more abundant in L V. When BOAA (10-6 M) was applied before, during and after TS, it did not block the induction of LTP, but did block the expression of LTP for as long as BOAA was present. Lower concentration of BOAA (10-7 M) had no effect on either induction or expression of LTP in L II. These data indicate that at other sites, the induction of LTP in motor cortex requires NMDA receptor activation. BOAA blocks the expression but not the induction of LTP in a concentration-dependent manner, probably through desensitization of the AMPA receptor. Supported by NS23807.

As part of an enkephalin analog program, a number of amides of (L)-2,6-dimethoxytryamine were synthesized and tested broadly. Since these compounds were superimposable on metazocine, testing included binding assays in sigma (3H-PPP) and phencyclidine (3H- TCP) assays. One compound, SC-48443, was found to bind potently, selectively, and competitively to the phencyclidine site. Structure-Activity data and physiological and behavioral results will be discussed.

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EXCITATORY AMINO ACIDS INCREASE INTRACELLULAR CALCIUM IN CULTURED PURKINJE NEURONS. D. L. Godin*, K. L. Parsons and M. Kieper, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037.

The effect of selective excitatory amino acid receptor agonists on intracellular Ca2+ levels of cultured cerebellar Purkinje neurons was examined using digital imaging techniques and the Ca2+ sensitive dye fura-2. Several agonists were tested: (a) the ionotropic receptor agonist AMPA, (b) the metabotropic receptor agonist (1S,3R)-1-aminoocyclopentane-1,3-dicarboxylic acid (ACPD), and (c) quisqualate (Qui), which activates both the ionotropic and metabotropic receptors. AMPA plus glycine and 100 μM strychnine were applied in high K+ saline applied in cultured Purkinje neurons. Several agonists were tested: (a) the ionotropic receptor agonist AMPA and domoate (Dom), (b) the metabotropic receptor agonist (1S,3R)-1-aminoocyclopentane-1,3-dicarboxylic acid (ACPD), and (c) quisqualate (Qui), which activates both the ionotropic and metabotropic receptors. Calcium measurements and electrophysiology of chick embryos (E16 to P2) for changes in intracellular calcium ([Ca2+]i) were measured at 3 sec intervals. In both the somatic and dendritic regions, Qui, AMPA and Dom elicited single channel currents in outside-out patches. Currents were recorded at 3 sec intervals. The open probability was reduced to 0.54±0.12, 0.78±0.12, and 0.89±0.16, respectively. Additionally, only one type of burst was detected with time constants of 2.6±1.45, 1.39±0.31, and 1.14±0.24 ms, respectively. These effects were voltage dependent, suggesting that these compounds act as open channel blockers at the NMDA receptor. Our findings indicate that the alkyd ring in quisqualic acid is more potent blockers of NMDA receptors than the mono-9-aminoacridines, THA. (Support: US Army Med. Res. & Dev. Comm. Contr. DAMD17-88-C-8119; USPHS Grant NS 25296)

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EXCITATORY AMINO ACIDS: RECEPTORS VIII


We have shown that 4-methylphenazole (4-MP) interacts with the NMDA receptor of the cultured hippocampal neurons, activating single channel currents that resembles those evoked by NMDA (Soc. Neurosci. Abs. 17: 1538, 1991). Applying the whole cell patch-clamp technique to cultured fetal hippocampal neurons from 18- to 20-day gestation rats, we further analyzed the interactions of 4-MP with the NMDA receptor and with the strychnine-sensitive glycine receptor. 4-MP (1 μM) blocks reversibly the strychnine-sensitive whole cell current activated by 50 μM glycine. Also, whole cell currents activated by 50 μM NMDA were blocked by the same concentration of 4-MP. In addition, biochemical studies were carried out in rat brain synaptosomes. 4-MP alone caused a very small increase in the basal Ca2+ uptake into the synaptosomes, but, it clearly reduced the uptake induced by 100 μM NMDA. Moreover, at Torpedo nicotinic receptors (nAChR), in the absence of carbamylcholine, 4-MP enhanced the binding of [3H]tetrodotoxin, an effect similar to that elicited by the nicotinic agonists. However, 4-MP did not displace α-bungarotoxin from its binding site, thus suggesting an allosteric activation of nAChR via a site distinct from that of ACh. These data indicate that 4-MP is recognized by different binding sites on several receptors, and that such effects could account for its therapeutic efficacy in the treatment of alcoholic intoxication.

**632.11**

IN VIVO STIMULATORY EFFECT OF GLYCINE IN STRIATUM AND HIPPOCAMPUS. G. Yaid, K. Facak, J.D. Harvey-White, L.J. Kopin* and D.S. Goldstein. Clinical Neuroscience Branch, NINDS, NIH/NIH Bethesda, MD 20892.

Glycine (G) is an excitatory neurotransmitter in the spiny cord and medulla. Binding to specific Gly receptors increases transmembrane Cl-conductance and hypotensive neurons. Striyxhine (Str) induced this effect. Gly's role in the release of glutamate in higher brain areas was examined by microdialysis in the striatum and the hippocampal CA3 region of freely-moving rats. After probe insertion, artificial CSF was infused for 20-30 h, and after 3 min basal microdialysate samples were obtained, the infused was changed to CSF containing Gly (0.02-20mM) with or without Str (10 μM). Gly produced dose-related increase in the release of dopamine (DA) and its metabolites in striatum and of norpinephrine (NE) and adenosine (AD) in hippocampus. Str blunted these increases in hippocampus (* P < 0.01) and shifted the dose-response curves to the right by about 1 log unit (Fig. 1). The results indicate a stimulatory effect of Gly on cholinomimetic release in the striatum and hippocampus, via Str-sensitive receptors.

**632.12**


The number of amino acid molecules required to react with a receptor to cause an excitatory response is not known. Experiments were performed on rat neostriatum and cortex using a 6-barrel microdialysis electrode assembly filled with glutamate, aspartate and DL-homocysteate.

The experiments were performed on rats anesthetized with urethane (1.5 g/kg). Extracellular recordings were obtained from the thalamocortical relay cells of the ventrolateral thalamus identified by their response to stimulation of hindlimb nerve and the somatosensory cortex.

Hill plots of log (amino acid currents) vs log (V/VO) where V is the ratio of the observed rate of firing to the maximum attainable rate were done on neurons tested with both glutamate and aspartate. The results yielded average slopes of 2.8 ± 0.2 SE (glutamate, n=17) and 3.4 ± 0.6 SE (aspartate, n=11).

This study suggests that more than one amino acid molecule must react with a receptor to cause its activation.

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**OPINATE RECEPTOR LIGANDS**

**633.1**


Etonitazene (ETZ) is a synthetic opiate approximately (3H)-naloxone binding (Port and Snyder, BD. Emsam. 10:688-697 1974), its affinity for the various known opiate receptor sites has not been determined. Accordingly, we measured the ability of ETZ, fentanyl, and morphine to compete with [(3H)-DADLE (+DPDPE), (3H)-DAGO (+DSLET), (3H)-DPDPE, (3H)-[N]-99,99, and (3H)-DADG]. In order to label mu, delta, kappa and sigma receptors, respectively, in membrane preparations from rat brain, ETZ had a 2500 fold greater affinity than morphine at the mu site, (3H)-ETZ = 0.00041nM; fentanyl = 0.007nM; morphine = 1.11 nM). Although ETZ competes with high affinity for (3H)-naloxone binding (Port and Snyder, BD. Emsam. 10:688-697 1974), its affinity for the various known opiate receptor sites has not been determined. Accordingly, we measured the ability of ETZ, fentanyl, and morphine to compete with [(3H)-DADLE (+DPDPE), (3H)-DAGO (+DSLET), (3H)-DPDPE, (3H)-[N]-99,99, and (3H)-DADG]. In order to label mu, delta, kappa and sigma receptors, respectively, in membrane preparations from rat brain, ETZ had a 2500 fold greater affinity than morphine at the mu site, (3H)-ETZ = 0.00041nM; fentanyl = 0.007nM; morphine = 1.11 nM). Although ETZ competes with high affinity for (3H)-naloxone binding (Port and Snyder, BD. Emsam. 10:688-697 1974), its affinity for the various known opiate receptor sites has not been determined. Accordingly, we measured the ability of ETZ, fentanyl, and morphine to compete with [(3H)-DADLE (+DPDPE), (3H)-DAGO (+DSLET), (3H)-DPDPE, (3H)-[N]-99,99, and (3H)-DADG]. In order to label mu, delta, kappa and sigma receptors, respectively, in membrane preparations from rat brain, ETZ had a 2500 fold greater affinity than morphine at the mu site, (3H)-ETZ = 0.00041nM; fentanyl = 0.007nM; morphine = 1.11 nM).

**633.2**

AFFINITY LABELING HUMAN OPIOID RECEPTORS WITH A NOVEL IODINATED DERIVATIVE OF NalBzoH. K.M. Standifer,* G.S. Ciszewska, I. Chong, I.Z. Ginos, J.L. Biedler, and G.W. Pasternak. Cotzias Laboratory of Neuro-Oncology and Laboratory of Cellular and Biochemical Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

The BE(2)-C clone of SK-N-BE(2) human neuroblastoma cell line contains high levels of opioid binding sites which were quite similar to levels in brain. Selective binding assays revealed the presence of mu, delta and kappa receptors in a 2:1:2 ratio. FPLC of CHAPS-solubilized [3H]-NalBzoH affinity-labeled receptors from these cells over a Mono-Q column demonstrates the presence of at least 5 separable peaks of labeling. Since its isolation was investigated using a 6-barrel microdialysis electrode assembly filled with glutamate, aspartate and DL-homocysteate, it may still be potent opiates. The 3-[35]-4-amino-benzyolhydrazine of naloxone (35)-NaAlAmBzoH) labels opioid binding sites on the BE(2)-C cells with high affinity (Kp 1 nM; Bmax 190 fmol/mg protein). The sensitivity of the binding towards DADL, DAMGO and DPDPE implies the labeling of mu, delta and kappa sites. USO.48SH does not compete binding, confirming the absence of kappa receptors in these membranes. Like [3H]-NalBzoH, [35]-NaAlAmBzoH can covalently label binding sites following exposure to UV light. SDS PAGE of labeled membranes reveals two major and two minor specific bands between 50,000 and 100,000 daltons which are also competed by low nanomolar concentrations of opioids.

TIPP was shown to be a selective δ opioid receptor antagonist using the guinea pig duodenum and mouse vas deferens bioassays (Scheldt et al., 1992 FASEB abstract #W999). A radioactive analogue of this peptide would be valuable for studies of the δ opioid receptor since it is more selective than naltrindole which is the only available δ receptor ligand whose affinity is suitable for radioligand binding assay. One approach to radiolaodination is radiosynthesis of the Phe ring using a diazonium intermediate (Sharma et al., J. Org. Chem. 56: 4981, 1991). The binding affinity of [3H]-TIPP was tested at sites labeled by selective opioid receptor radioligands for this reason.

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>TIPP IC50 (nM ± SEM)</th>
<th>[3H]-Phe-TIPP IC50 (nM ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ Selective naltrindole</td>
<td>29.9 ± 1.4</td>
<td>14.4 ± 0.7</td>
</tr>
<tr>
<td>μ Selective CTOP</td>
<td>154,600 ± 19,800</td>
<td>53,525 ± 9,375</td>
</tr>
<tr>
<td>κ Selective U-69593</td>
<td>160,900</td>
<td>not determined</td>
</tr>
</tbody>
</table>

The results show that iodination of the Phe aromatic ring preserves most of the δ opioid receptor binding affinity and selectivity of the parent peptide. Radiolabeled TIPP is being prepared and its binding properties for δ opioid receptors studied. Supported in part by NIDA grants.

633.4 SELECTIVE LABELING AND RESOLUTION OF KAPPAN RECEPTOR SUBTYPES BY [125I]IOXY. Q. Jiang*, J.S. Partilla, H. Xu, B.B. de Costa, K.C. Rice* and R.B. Rothman. NIDA Addiction Research Center, PO Box 5180, Baltimore, MD 21224. 6363, NIDDK, NIH, Bethesda, MD 20892.

Previous work demonstrated that, using membranes depleted of μ and δ sites by pretreatment with BIT and FIT, [3H]bremazone labels two populations of μ binding sites termed μ1 and μ2, whose anatomical distribution and ligand-selectivity pattern differs from that of μ and δ binding sites. The present study was undertaken to characterize μ2 binding sites with the novel antagonist ligand, [125I]IOXY. [125I]IOXY (SA=2200 Ci/mmol) was prepared by iodination of BB669, deacetylation, and purification by HPLC. Assays were conducted for 4 to 6 hr at 4°C in 50 mM TRIS-Cl, pH 7.4, and 10 mM NaCl. Binding surfaces generated with (1)-(12,5)-USO, DMAPG and [Leu5]enkephalin (LE) (274 nM) were fit to one- and two-site binding models. The two site model fit the data considerably better than a one-site model (p<0.001). The two sites fit resolved two sites, present at relative concentrations of 0.64 (μ1) and 0.44 (μ2). The Kd/Ki values (nM) for the drugs at the two sites were: IOXY (0.46, 0.73), (1)-(12,5)-USO,488 (3527, 5051), DMAPG (11.9, 1262) and LE (57.7, 12295). Structure-activity studies (in progress) indicate that the two sites labeled by [125I]IOXY are the same as the two sites labeled by [3H]bremazone. Viewed collectively, these data provide further evidence for the existence of subtypes of the μ2 binding site.

633.5 NALTRINDOLE BENZOFURAN IS NOT A κ-OPIOID SELECTIVE ANTAGONIST IN THE RAT SPINAL CORD. P.E. Stewart* & D.L. Hammond. Dept. of Anesthesia and Critical Care, The University of Chicago, Chicago, IL 60637.

Recent studies with the κ-selective opioid antagonist Naltrindole support the presence of δ-mediated antinociception in the spinal cord of the mouse and the rat. Naltrindole benzofuran (NTB) is also reported to be a δ opioid receptor antagonist in the mouse. This study examined the selectivity of NTB in the rat spinal cord using the tail-flick (TF) and hot plate (HP) tests. Sprague-Dawley rats were injected intrathecally (i.t.) with the δ opioid agonist DPDPE or the κ-selective agonist [D-Ala2,MePhe4,Gly-ol]-enkephalin (DPDPE) and DAMGO each dose-dependently increased TF and HP latencies. NTB (3-10 μg i.t.) dose-dependently antagonized the increase in TF and HP latencies produced by DPDPE. The antagonism did not appear to be competitive. The increase in TF and HP latencies produced by DAMGO was also antagonized by these same doses of NTB. The antagonism was not competitive. Similar results were obtained with s.c. administered NTB (10-30 mg/kg). These data suggest that NTB is not a selective δ opioid antagonist in the spinal cord. Supported by PHS Grant DA 06736.


We have prepared a series of rigid tetracyclic analogs of 3-PPP, dopamine and other neuroreceptor ligands, namely octahydrophenoanthroquinolines (OHNQs) (structure shown below). Each OHNQ exists as four diastereomers which are topologically very different. These compounds differ from conformationally flexible analogues which have activity at sigma receptor binding sites (SRBSs). D2 and other neuroreceptor sites. Binding data show that OHNQs have modest to high affinities to SRBSs in mouse cerebellar homogenates labeled with [3H]D2 or [3H]DTG, and have low affinity toward D3 receptors in bovine striatal preparations. Additional biological activity has been seen toward the NOVASCREEN program. The structure-activity relationships of these compounds toward different neuroreceptor systems will be presented. These compounds are synthesized in three steps from corresponding C-tetralones; the key step involves reductive ring closure by high pressure hydrogenation with Raney nickel. All four diastereomers synthesized, have been assigned by X-ray crystallography and 13C NMR spectroscopy.


Opioid effects of N-cyclopropylmethyl-NOR-14β(Bromoacetamido)-7,8-dihydomorphinone (N-CPM-H2BAMO) were investigated in the mouse 55°C tail-flick assay. Mice (20-30g) were dosed orally with N-CPM-H2BAMO (1 mg/kg) or saline as vehicle control. The antinociceptive effect of N-CPM-H2BAMO lasted up to 1 hr, with a maximal effect at 10 min after i.c.v. administration. The antinociceptive ONP value (50% of vehicle control) was 40% at 10 min. N-CPM-H2BAMO produced a dose- and time-dependent antinociception. The antinociceptive effect of N-CPM-H2BAMO in the writhing assay) failed to prevent the agonistic effect of N-CPM-H2BAMO in the writhing assay) failed to prevent the increase in TF and HP latencies produced by Dynorphin A-(1-13), which is a potent and selective Dyn A related peptide without motor effect at analgesic doses. Supported by MRC.


Dynorphin A-(1-13)-Tyr-Leu-Phe-Asn-Gly-Pro, (Dyn la), was previously shown to be a highly potent and selective κ opioid peptide. Four analogues of Dyn la have been synthesized by the solid-phase procedure: #1: [9-CH2NH3+]Dyn la, #2: [9-CH2NH3+]-D-Leu[Dyn la, #3: 3-[MeTyr], 9-CH2NH3+]-Dyn la and #4: [MeTyr], 9-CH2NH3+, D-Leu[Dyn la. The peptides were purified and compared with Dyn la for their ability to compete with the binding of selective κ, μ and δ opioid ligands and to display antinociceptive activity in an acetic acid induced writhing test. All synthetic compounds displayed a high affinity for the κ receptor (Ki against [3H]EKC binding 0.5-1.8 nm) but compounds #3 and 4 were more selective. Compound #2 possessed the highest antinociceptive activity (AD50: 15.7 nmol/mouse) and the lowest motor effect (convulsion; CD50:15.4 nmol/mouse). Its κ selectivity ratio (1.5:7.12:9.6) was comparable to that of Dyn A-(1-13) (1.4:30). Compound #2 is thus a potent and selective Dyn A related peptide devoid of motor effect at analgesic doses. Supported by MRC.
633.9
CHRONIC INTRACEREBROVENTRICULAR INFUSION OF THE ANTI-OPIOID PEPTIDE, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH₂ (NPFF), DOWN-REGULATES MU OPIOID BINDING SITES IN RAT BRAIN. R.B. Rothman1, L.S. Grady2, H.Xu2, and J.L. Long3. 1NIDA Addiction Research Center, Baltimore, MD 21224. 2LNP, NIMH, Bethesda, MD 20892. 3Dept. of Med. Neurosci., WRAIR, Washington, DC 20307-5100.

NPFF, an endogenous mammalian anti-opioid peptide, has been shown by other laboratories to cause 1) the acute antinociceptive effects of morphine, 2) the development of morphine tolerance, and 3) naloxone-induced withdrawal in morphine dependent rats. The present study determined the effect of chronic NPFF on mu opioid receptors, and mRNA for the endogenous opioids dynorphin and enkephalin. Rats received i.c.v. infusions of either saline or NPFF (5 µg/μl) for 13 days via ALZET 2002 osmotic minipumps. Homogenate binding studies, which used whole brain membranes, demonstrated that NPFF decreased the Bmax of mu binding sites (labeled by [3H]DAMGO) from 262±12 to 192±12 fmol/mg protein, and increased the Kd from 1.1 nM to 2.3 nM. Quantitative receptor autoradiography and in situ hybridization experiments were conducted with sections collected at the level of the striatum. The density of mu opioid binding sites labeled by [3H]DAMGO was decreased in all brain areas except the corpus callosum, and there was no change in the mRNA for dynorphin or enkephalin in caudate, the motor cortex, or the hippocampus. Studies in progress are examining the effect of chronic i.c.v. NPFF on the acute antinociceptive effects of morphine, as well as the development of morphine tolerance and dependence.

633.11

Our goal was to develop an automated, high-capacity functional assay in vitro that would report on the intrinsic efficacy of opioids. To this end, we characterized the inhibitory effects of opioids on prostaglandin E₁ (PGE₁)-stimulated formation of cyclic adenosine monophosphate (cAMP) in NG108-15 neuroblastoma x glioma (NG) cells. The NG cells were pre-incubated with diverse structural classes of opioids for 15 minutes in assay buffer containing IBMX, a phosphodiesterase inhibitor. We then measured the activities of the opioids for inhibiting prostaglandin E₁-stimulated cAMP formation. The cAMP was measured by RIA methods involving a charcoal-based separation, or the newer scintillation proximity assay. Validation of this assay was accomplished by comparing the EC₅₀ values obtained in NG cells to those obtained in the standard in vitro assay; opioid-elicited inhibition of electrically-stimulated contractions in mouse vas deferens. Our method correlated with the more traditional, labor-intensive, animal-based assay. We are applying these methods to other bioassays.

633.12
EXPRESSION OF c-fos & PREPROENKEPHalin (PpeK) mRNA IN HUMAN NEUROBLASTOMA SK-N-BE(2) CELLS. J. Cheng*, Y. Zhu, C.E. Inturrisi, K.M. Standlee, J.L. Bielder, C.W. Fastenak. The Corazon Laboratory of Neuro-Oncology & Laboratory of Cellular and Biochemical Genetics, Memorial Sloan-Kettering Cancer Center and Departments of Neurology & Neurosurgery and Pharmacology, Cornell University Medical College, New York, NY 10021.

The human neuroblastoma clonal cell line BE(2)-C from the SK-N-BE(2) line expresses functional mu, kappa, and delta opioid receptors. Binding studies reveal total binding levels similar to brain with a mu:kappa:delta distribution of 2:1:1. In addition, selective agonists for each receptor subclass inhibit forskolin-stimulated cAMP accumulation. We now demonstrate that this cell line also expresses mRNA for opioid peptides and c-fos. Using solution hybridization, we quantitated the levels of PPeK mRNA and c-fos mRNA in BE(2)-C cells and their regulation by forskolin. The basal level of PPeK mRNA equivalents is 0.14 ± 0.04 pg/cell RNA using a 32P-labeled riboprobe transcribed from a rat cDNA. Preprodynorphin and c-fos mRNAs are also measurable in these cells. Cells exposed to forskolin (100 µM) for 2 hr showed a 4-fold increase in c-fos mRNA levels. This elevation returns to basal levels by 6 hr. This same forskolin treatment also increases PPeK mRNA levels, but far more slowly than c-fos. Thus, BE(2)-C cells contain multiple classes of opioid receptors as well as mRNAs of several opioid peptides and the c-fos proto-oncogene and should prove useful for the study of opioids and their receptors.

633.13
DIFFERENTIAL EFFECT OF CHRONIC MORPHINE TREATMENT ON THE EXPRESSION OF G-PROTEIN AND ENDOGENOUS OPIOID PEPTIDES IN ADULT RAT BRAIN. E.R. Davis and A. Tempel*. Lab of Neurochemical Pathophysiology, Sloan-Kettering Cancer Center and Departments of Neurology & Psychiatry, Sloan-Kettering Cancer Center and Departments of Neurology & Psychiatry, Albert Einstein College of Medicine, Bronx, NY 10461.

The molecular mechanism involved in the development of opiate tolerance and dependence is still unclear. The majority of studies have failed to show any correlative alterations in receptor density in adult rats following chronic morphine treatment. In this study, we examined the influence of morphine to attenuate 1) the acute antinociceptive effects of morphine, 2) the development of morphine tolerance, and 3) naloxone-induced withdrawal in morphine dependent rats. The present study determined the effect of chronic morphine on mu opioid receptors, and mRNA for the endogenous opioids dynorphin and enkephalin. Rats received i.c.v. infusions of either saline or morphine (5 mg/kg) for 13 days via ALZET 2002 osmotic minipumps. Homogenate binding studies, which used whole brain membranes, demonstrated that morphine decreased the Bmax of mu binding sites (labeled by [3H]DAMGO) from 262±12 to 192±12 fmol/mg protein, and increased the Kd from 1.1 nM to 2.3 nM. Quantitative receptor autoradiography and in situ hybridization experiments were conducted with sections collected at the level of the striatum. The density of mu opioid binding sites labeled by [3H]DAMGO was decreased in all brain areas except the corpus callosum, and there was no change in the mRNA for dynorphin or enkephalin in caudate, the motor cortex, or the hippocampus. Studies in progress are examining the effect of chronic i.c.v. morphine on the acute antinociceptive effects of morphine, as well as the development of morphine tolerance and dependence.
**634.1**

**TOPOGRAPHICAL ORGANIZATION OF MIDBRAIN DOPAMINE AFFERENTS TO THE MEDIAL PREFRONTAL CORTEX OF THE RAT.**

A. Boudreau* and A. V. Deutch. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06510, and VA Medical Center, West Haven, CT 06516.

We have previously demonstrated that the responsiveness of the prefrontal cortical dopamine (DA) innervation to mild stress differs across the different closely-spaced topographical fields of the medial prefrontal cortex (PFC). This may be due to either distinct populations of midbrain DA neurons projecting to different parts of the PFC, or alternatively to changes restricted to the rostral terminal region (previously regulation of DA release). We therefore examined the distribution of midbrain DA neurons projecting to the PFC by placing small isotope-containing deposits on fluorogold (FG) in the PFC, and dual-labeling for FG and tyrosine hydroxylase (TH).

Retrogradely-labelled (FG-positive) cells were most frequently seen in the rostral VTA (supramammillary region). FG labelled neurons were next most frequently observed in the nuc. parabrachialis pigmentosus (PBP), followed by the caudal linear (CL) and then the rostral linear (RL) nuclei. Only rarely were cells seen in the interstitialis and paragnial nuclei.

More medial injections of the PFC (i.e., infralimbic and prelimbic) resulted in more double-labelled (FG + TH) cells in the medial VTA (RL and CL). More laterally placed injections resulted in more double-labelled cells in more lateral portions of the VTA (PBP). There also appeared to be a rough anatomo-topographical gradient. However, our preliminary data suggest only a crude topographical organization, one that may not be consistent with separate populations of midbrain DA neurons innervating different portions of the PFC. Supported by MH-45124 and the West Haven VA.

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**634.2**

**IN VIVO SPECT COMPARISON OF THREE HIGH AFFINITY RADIOLIGANDS FOR DOPAMINE D2 RECEPTOR.**


Yale University/VA Medical Center, West Haven, CT 06516, University of Pennsylvania, Philadelphia, PA, Vanderbilt University, Nashville, TN, Sumitomo Chemical Co., Osaka, Japan and Kyorisu University, Japan.

The regional distribution and pharmacological specificity of iodobenzofuran (IBF), epidepride (EPID) and 2'-iodospiperone (2'-ISP) were measured with serial SPECT scans in non-human primates. A series of 13 IBF, 6 EPID and 2'-ISP studies were conducted on 6 ovariectomized female baboons (9-13 kg) under isoflurane anesthesia. Animals were injected with 5-16 mci I-131-labeled agent i.v. and scanned in the Strichman 810X Imager. All three ligands showed specific striatal uptake which reached peak at 37, 140 and 55 min postinjection (PI) for IBF, EPID and 2'-ISP, respectively, with striatal ratios to nonspecific (cortex or cerebellum) 3.12 and 2.2, these ratios increased to 10,19, and 3.4 after 4 hr. Ex vivo autoradiographic studies in one animal for each ligand sacrificed at 30, 120 and 80 min PI demonstrated the highest uptake in the caudate and putamen, and striatum/cortex ratios were 6,10, and 5, respectively. From the peak striatal activities, washout rates for those ligands were 32, 8 and 15%/hr. IBF and 2'-ISP plateaued for 30-50 min while EPID for 2 hr, followed by a steady but gradual washout over a period of 3 hr. Raclopride (1 mg/kg) produced complete displacement from striatum of all agents with washout rates increased to 126%, 61% and 38%/hr. Riluzerin and apomorphine (1 mg/kg) had no significant effect on striatal washout rates.

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**634.3**

**DOPAMINE AUTORECEPTOR ANTAGONIST AJ76 DISRUPTS AUDITORY SENSORY GATING IN RATS.**


The latency auditory evoked potential recorded to the second of a closely-spaced (0.5 sec interval) pair of clicks is reduced as compared to the first, in unmedicated rats and in normal humans. Administration of amphetamine to rats or humans disrupts this "gating", producing a schizophrenia-like pattern of response. Similar alterations in gating have been elicited by elevation of endogenous norepinephrine levels through administration of yohimbine, a presynaptic alpha, selective adrenergic antagonist. To determine if elevation of endogenous dopamine levels through blockade of the autoreceptor would also produce alterations in sensory gating, the dopamine receptor antagonist AJ76 was administered at a dose thought to act only presynaptically (3.5 mg/kg, sc). AJ76 was administered to rats with chronic indwelling recording electrodes located on the brain surface at "vertex". At 20 and 45 min post injection, AJ76-treated rats showed a loss of sensory gating as compared to unmedicated trials. This was due to an increase in the amplitude of the N40 wave in response to the second (test) stimulus with no change in the amplitude of the response to the first (condition) stimulus. There were no significant changes observed at 65 min post injection. Thus, increases in endogenous dopamine levels can impair sensory gating in rats. (Supported by P50 MH44212-03.)

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**634.4**

**ANTAGONISM OF COCAINE, AMPHETAMINE AND OTHER DOPAMINERGIC STIMULANTS BY THE PREFERENTIAL DOPAMINE AUTORECEPTOR ANTAGONIST (+)-UH232 IN THE INTRACRANIAL SELF-STIMULATION ACTIVITY MODEL.**

Torben Kling-Peterson*, Elizabeth Liang and Kell Synness. Dept of Pharmacology, Univ. of Goteborg, P.O.B 305 31, 400 30 Goteborg, SWEDEN.

The preferential dopamine autoreceptor antagonist (+)-UH232 exerts a weak stimulatory effect when tested in locomotor activity experiments using habituated animals. However, (+)-UH232 also blocks d-amphetamine, cocaine, and apomorphine-induced hyperactivity, and the behavioral effects of (+)-UH232 appear to be dependent upon the baseline activity of the animal.

Various behavioral models have been utilized in order to investigate the possible reinforcing properties of (+)-UH232. In the intracranial self-stimulation (ICSS) paradigm in the rat, bipolar electrodes aimed at the median forebrain bundle delivers mono-phasic, cathodal current of varying intensities. By establishing a threshold value (categorical ESC10) using a rate/intensity-model, results from different experiments can be statistically compared. (+)-UH232 produced a weak inhibitory effect over a wide dose range (1-16 mg/kg). Caffeine (1-16 mg/kg sc) and d-amphetamine (0.25-1 mg/kg sc) on the other hand produced a clear lowering of the ECS10 values, indicating stimulatory effects. (+)-UH232 (16 mg/kg sc) blocked the stimulatory effects of both cocaine (4-16 mg/kg) and d-amphetamine (0.25-4 mg/kg). It is possible that there is a qualitative difference in the antagonism of these two dopamine stimulants.

We are presently investigating the interactions of (+)-UH232 with the dopamine reuptake inhibitor GBR-12909 and DA D2 receptor agonist quinpirole in the ICSS paradigm and locomotor activity model. (Supported by The Upjohn Company, Kalamazoo, MI.)

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**634.5**

**DESTRUCTION OF MESOLIMBIC DOPAMINE NEURONS BY INTRA-VTA INJECTIONS OF 6-OHDA DOES NOT BLOCK THE LCOMOTOR ACTIVATING EFFECTS OF AMPHETAMINE.**


Recently we reported that while acute systemic injections of nicotine substantially increase dopamine (DA) utilization in the nuc. accumbens (N. Acc.), repeated these injections abolishes this effect even though this drug's locomotor effects become enhanced (Vézina et al., J-PET, in press). This suggests that nicotine may elicit at least some of its locomotor effects via not mesolimbic DA neurones. In the present study, this hypothesis was investigated by assessing the locomotor response to nicotine following destruction of the mesolimbic DA system. Rats received bilateral injections of 6-OHDA (4mg/kg/ul) into the ventral tegmental area (VTA). Four weeks later, the locomotor response of these animals to (+)-nicotine bitartrate (0.4 mg/kg, base, s.c.) was compared to that of non-lesioned controls. Depletions of N.Acc. DA of up to 100 % of control concentrations did not block the acute locomotor response to nicotine. These depletions also did not prevent the progressive enhancement of nicotine's locomotor effects when injections were repeated daily for seven days. The results, together with others demonstrating that such 6-OHDA-induced depletions of N.Acc. DA completely block the locomotor response to amphetamine up to 33 days post-lesion, extend our earlier findings with N.Acc. DA utilization and suggest that mesolimbic DA is not necessary for the elicitation of locomotor activation by nicotine.

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**634.6**

**CENTRAL CORTICOSTEROID RECEPTORS AND STRESS RESPONSIVENESS IN TWO PHARMACOGENETICALLY SELECTED RAT LINES.**

J. A. M. Van Vliet* and C. A. Stock*.


Two rat lines derived from a normal outbred Wistar population were selected on their susceptibility to the dopamine antagonist apomorphine. The drug susceptible (apo-sus) rats are characterized by a fleeing response following defeat, whereas the apo-unus rats freeze. This differentiates one suggest a different responsiveness of apo-sus and apo-unus rats to stress. The hypotalamic-pituitary-adrenal (HPA) activity in response to a conditioned emotional stimulus (CER) was determined by blood collection from chronically cannulated rats over 4 hours following CER. In apo-sus rats, CER-induced plasma ACTH and basal plasma ACTH were elevated. However, this rat line did not show increased stress-induced or basal plasma B levels. Apo-sus rats also displayed a more pronounced neuroendocrine response to HPA-stimulation with exogenous CRF. Apo-sus and apo-unus rats show differences in central AR and B as studied in situ hybridization and ligand binding assays. Thus, pharmacogenetically selected rats with different functional adrenergic activity in the brain reveal enhanced reactivity of the corticosteroid controlled HPA-system.
634.7 NOVELTY OR FAMILIARITY DIFFERENTIALLY AFFECTS IN VIVO STRIATAL/SEPTAL D2 RECEPTOR BINDING IN ISOLATED NEONATAL RATS. K.M. WARD and P. KROHE* Trinity College, Psychology, Hartford, CT 06106. Infants separated from their caretaker emit behavioral and physiological responses associated with stress (Krohe et al. 1991). Neurochemical mediation of these responses may be affected by the novelty of the isolation environment. Dopamine has been shown to control the neural circuitry responsible for the behavioral changes observed in these infants, with cAMP-mediated signaling playing a role in the regulation of dopamine release. This system has the potential to rapidly assess drugs for their antidepressant action, and may provide a new model for human antidepressant drug screening.

634.8 METABOLIC MAPPING OF THE "PRIMING" PHENOMENON IN RATS BEARING UNILATERAL 6-HYDROXYDOPAMINE LESION OF THE NIGROSTRIAL PATHWAY. L.E. Fuster*, M. Morelli, R. Terent and G. Di Chiara. Dept. Neurosciences, Università "La Sapienza", Rome, Italy; and Neuropharmacology, University of Cagliari (Italy).

In rats bearing unilateral 6-hydroxydopamine (6-OHDA) lesion of the dopaminergic nigrostriatal neurons, "priming" with a single administration of the D2 agonist, LY-71555 (0.3 mg/kg, sc), strongly potentiates the behavioral changes observed after a second D1 agonist treatment induced by the D1 agonist, SKF-38393 (5.5 mg/kg, sc). The 2[14C]-deoxyglucose (DG) method for the measurement of local cerebral glucose utilization (LCGU) was applied to identify the several substrates involved in the priming phenomenon. Lesioned animals received two injections, three days apart, as follows: Group 1: saline/saline; Group 2: LY-71555/saline; Group 3: saline/SKF-38393. The DG experimental procedure was begun 28 minutes after administration of the drug or vehicle. Unilateral 6-OHDA lesion per-se (Sal-Sal) produced increases in LCGU in the globus pallidus (GP) and the ventral bridge (vb) area but not in the caudate nucleus (SNr) or globus pallidus externa (GPe) region. Rates of glucose metabolism in LS/Sal treated rats were similar to those measured in the vehicle-treated animals. Single administration of SKF-38393 (Sal-SKF) abolished the lesion-induced metabolic asymmetry in the LH but did not have any effect on the GP. Furthermore, it increased LCGU in the substantia nigra pars reticulata (SNr) of the lesioned side. LY-SKF treatment produced marked metabolic asymmetries by increasing LCGU in the SNr and entopeduncular nucleus (EPN), and decreasing it in the LH of the lesioned side. These changes were significant also when compared to the corresponding values of the other experimental groups. Again, LY-SKF failed to modify the lesion-induced metabolic asymmetry in the GP. These results indicate that the behavioral changes observed after SKF-38393 in primed rats are associated with a marked enhancement of the metabolic response in the SNr, EPN and LH and suggest that priming exerts a facilitatory influence on the ability of D1 receptors to stimulate the striato-nigral and striato-entopeduncular pathways.

634.9 INTRAPERITONEAL SALINE INJECTION DECREASES DARPP-32 (DOPAMINE AND CYCLIC AMP-REGULATED PHOSPHO PROTEIN) OF M3 = 32,000) mRNA LEVELS IN SPECIFIC REGIONS OF MOUSE BRAIN. R. M. Lewis* and R. G. Perez. Dept. of NACS, University of Pittsburgh, Pittsburgh, PA 15261.

DARPP-32 is a cytosolic phosphoprotein phosphatase inhibitor that is phosphorylated by cyclic AMP-dependent protein kinase in response to dopamine. Phosphorylated DARPP-32 down-regulates Na+K+ - ATPase. Calcium dephosphorylates DARPP-32 in response to NMDA. DARPP-32 is enriched in regions of the brain that have D1 dopamine receptors. Neither expression of DARPP-32 during development of the brain, nor maintenance of DARPP-32 levels in the adult appears to require dopamine. We tested whether excess dopamine could down-regulate the expression of DARPP-32 mRNA. Mice were injected daily with 0.5 ml of 0.9% NaCl (i.p., 30’ after 50 mg/kg Ro 4-4602 in 0.5 ml of 0.9% NaCl, i.p.) for one to five days. Uninjected mice served as controls. Mice were sacrificed six hours after the last injection. Levels of DARPP-32 mRNA were assessed by hybridization in sagittal sections of brain. The amount of DARPP-32 mRNA in layer VI of cortex, piriform cortex, and anterior olfactory nucleus of mice injected only with saline was lower than the amount of DARPP-32 mRNA in uninjected mice. No other regions of the brain were affected. Injection of 100 or 200 mg/kg L-DOPA in saline had the same effect as saline alone. We are testing each parameter to determine the cause of this decrease in DARPP-32 mRNA levels in specific brain regions.

634.10 EVIDENCE FOR COUPLING OF RAT SUBSTANTIA NIGRA (SN) DOPAMINE D1 RECEPTORS TO PHOSPHOSTROPHIN (PI) HYDROLYSIS. L.P. Marinic* and B.A. Waszczak. Pharmacology Section, Northeastern University, Boston, MA 02115.

We have previously demonstrated that the dopaminergic D1 agonist SKF-38393 and the D2 agonist SKF-634.9 abolishes the lesion-induced metabolic asymmetry in the LH but did not have any effect on the GP. Furthermore, it increased LCGU in the substantia nigra pars reticulata (SNr) of the lesioned side. LY-SKF treatment produced marked metabolic asymmetries by increasing LCGU in the SNr and entopeduncular nucleus (EPN), and decreasing it in the LH of the lesioned side. These changes were significant also when compared to the corresponding values of the other experimental groups. Again, LY-SKF failed to modify the lesion-induced metabolic asymmetry in the GP. These results indicate that the behavioral changes observed after SKF-38393 in primed rats are associated with a marked enhancement of the metabolic response in the SNr, EPN and LH and suggest that priming exerts a facilitatory influence on the ability of D1 receptors to stimulate the striato-nigral and striato-entopeduncular pathways.


The pigments containing cells of certain lower invertebrates have the ability to aggregate, while increases, produced by exposure to low levels of light or levels, produced by exposure to low doses of melatonin, induce pigment dispersion. Compared to the normal conditions, in the presence of these stimuli, the cells are more sensitive to less sequal dopamine receptor occupation in contrast to novelty which produces the opposite effect based presumably on the release of dopamine at these terminals.


The recently cloned D3 receptor has been shown to bind D2 receptor ligands when expressed in CHO-K1 cells (Seikoff et al. 1992). Whether this receptor can mediate the ability to transduce signals, is not known. In the present study, we report that D3 receptors, similar to D2 receptors, may function in this manner. Using a competitive radioligand binding assay, we have shown that the agonist quinpirole demonstrated a 113-fold greater affinity for D2 vs. D3 receptors. Additionally, both Seikoff et al. and Gehlert et al. (1992) report that D2 receptors, unlike D3, or dopamine, do not shift to a lower affinity state in the presence of GTP or analogues such as Gpp(NH)p. Previously, we sought to examine the D3-like pharmacology of receptors located in limbic and forebrain areas of the rat brain. The rat substantia nigra (SNpr) neurons. This excitatory response was lost after striatal lesions or injections of the precursor mepacrine EEDO injected in the SN, suggesting that D1 receptors in the SN are involved in the nigrostriatal terminals. Further studies indicated that adenylate cyclase might not mediate the D1 agonist effect. Specifically, iontophoresis of cAMP analogues did not mimic the agonist effect, whereas intranigral injection of pertussis toxin (PT), an inactive of Gi and Go proteins, completely abolished the excitatory response to SKF. An ADP ribosylation assay on SN punches taken from PT-treated rats revealed a 64% decrease in the ability of niger G-proteins to incorporate [32P]-NAD, confirming success of the PT injections. These results were surprising since D1 receptors have traditionally been thought to act through Gi to stimulate adenylate cyclase. Consequently, we were prompted to investigate whether PI hydrolysis might provide a second messenger coupling mechanism for the nigral D1 receptor. For these experiments, the SN and striata from 5 rats were used. Pooled slices were prepared with a tissue chopper, and then incubated in 1.7 μM myo-inositol for 1 hour. Aliquots of packed slices were incubated for 30 min with or without agonist (300 μM SKF). 3H-Inositol phosphates (3H-I-Ps) were quantified by anion exchange HPLC. Preliminary results revealed a 4-fold higher basal level of 3H-I-Ps in SN than striatum (per mg protein). Moreover, SKF appears to stimulate production of total 3H-I-Ps in both nigra and striatum to a similar extent (56 and 71%, respectively, n=4). These findings, while preliminary, support the possibility that SN receptors involved in the excitatory effect of D1 agonists on SNpr neurons may be coupled to the PI second messenger pathway. (Supported by NS 23541)
634.13  

DA neurons normally display a spontaneous activity which is irregular and alternates between a single-spoke mode of discharge and one which includes bursts of action potentials. In an attempt to better understand the ionic currents which may be responsible for maintaining these distinct patterns of activity, we have begun to study the inward calcium currents present in the cell body of mesencephalic dopamine neurones maintained in culture. Whole-cell patch-clamp studies were conducted using whole-cell patch-clamp methods. DA neurones were identified and studied in cultures which were 12-21 days old with the following procedure: cultures were grown in serum-free medium containing 10% ascorbic acid and 25 μM 5,7-dihydroxytryptamine. DA neurones were found contain three types of calcium currents. A Type current was observed which exhibited a low threshold for activation (-50 mV), displayed rapid inactivation and was partially blocked by both external application of either amlodrine or Nρ. A classic L-type calcium current was observed which could be activated from a holding potential of -40 mV, slowly and incompletely inactivated and was extremely sensitive to nifedipine. Finally, a N-type current was observed which was activated at the same threshold as the L-Type current but required prior hyperpolarization of the membrane, was transient and was blocked by external application of omega-conotoxin. DA (50-100 μM) stimulation of the D2 autoreceptors present on these cells resulted in a significant reduction in both L- and N-type calcium currents observed. Studies on the signal transduction pathways involved in mediating this autoreceptor modulation of calcium currents are currently in progress.  

(MH41557) (LAC), NS26081 (GRK)

634.14  
COUPLING OF D2-SHORT RECEPTOR ISOFORM TO ION CHANNELS. M.A. Castellano,* L-X Liu,† F.J. Monsma, Jr.,* D.R. Sibley,‡ L.A. Chiodo,* and G. Kapatos.* †Dept. Psychology, Univ. La Laguna, Canary Isl. (Spain), ‡Pharmacology, Wayne State Univ., Detroit, MI 48201.

Two isoforms of the dopamine (DA) D2 receptor, termed D2-short (D2S) and D2-long (D2L), have been discovered. Because both forms display the same pharmacological characteristics and are present in the brain regions that show robust response to DA, it is impossible to study their individual signal transduction mechanisms. To begin such an analysis, NG108-15 neuroblastoma-glial hybrid cells transfected to stably express D2S were used to investigate the coupling mechanism involved with the whole-cell patch technique. Transfected NG108-15 cells maintained in G4-18 were found to express inward currents mediated by both T- and L-type Ca++ channels, as defined by voltage-dependence of activation, rate of inactivation and sensitivity to antagonists. Pressure application of DA (100 μM) or the D2 agonist quinpirole (QUIN 100 μM) reduced both T- and L-type currents. Application of DA or QUIN also reduced the amplitude of a Ca++ dependent outward current. This latter effect was blocked in a concentration-dependent manner by inclusion of the Ca++-chelator BAPTA in the pipette solution, was not altered by CaCl2 in the bath solution and could be mimicked by pressure application of thapsigargin (10 μM). These effects were blocked by the D2 receptor antagonist eticlopride and were not observed in nontransfected cells. These results suggest that Ca++ mobilized from intracellular stores is involved in the reduction of the K+ current by D2S receptor stimulation. The inhibitory effect of D2S stimulation on two distinct Ca++-dependent inward currents and a K+-dependent outward current suggests that a common mechanism, possibly mediated by mobilization of intracellular Ca++, may be involved. Similar studies of NG108-15 cells transfected with the D2L form are currently in progress and should determine when these receptors are coupled to ion channels.  

(NS26081, MH41557)

634.15  

Dopamine (DA) autoreceptors are known to be critically involved in the regulation of the physiology of mesencephalic DA neurones. Recently, we have observed three different K+ currents present in these cells, IA, IK, and INaK are modulated by stimulation of the D2 DA autoreceptor. Because it is known that DA receptors belong to the larger superfamily of G-protein-coupled receptors, we examined the signal transduction pathways involved in mediating this modulation. Whole-cell patch-clamp techniques were used to compare these different currents in DA cells maintained in culture. All three currents were significantly reduced by stimulation of DA autoreceptors with either DA or quinpirole (50-100 μM). Preincubation of the DA cells with pertussis toxin (500 ng/ml for 4-5 hrs) completely blocked the DA autoreceptor modulation of all three K+ currents. Application of 100 μM GDPβS also blocked the DA modulation of these currents while intracellular application of GTPγS mimicked the activation produced by DA. In addition, the application of a polyclonal antibody that specifically recognizes Go1α subunits (antiserum 3, Gramann and Kapatos, J. Neurochem. 54:1995, 1990) completely blocked the ability of DA autoreceptors to modulate these potassium currents while the preimmune serum was without effect. Taken together, these findings demonstrate that IA, IK, and INaK currents are increased by DA autoreceptor activation via a common mechanism which involves modulation by the Go1α subunit of the associated K+ channel proteins.  

(MH41557) (LAC), NS26081 (GRK)

634.16  
DIETARY PROTEIN MODULATES DOPAMINE LEVELS IN THE RAT BRAIN. J. Brock, S. Faroqui, E. S. Osvaly, A. Basoli, and C. Prasad. Pennington Biomedical Res. C., Baton Rouge, LA, 70808; Dept. Medicine, LSU MC, New Orleans, LA, 70112.

Rats that consume 50% protein exhibit hyperactivity and hyperresponsiveness to nociceptive stimuli, in which facilitation of dopaminergic activity has been implicated. We studied the regional distribution of dopamine (DA) and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in brains of rats maintained on high-protein (50%), low-protein (20%), and low-protein (8%) diets for 36 weeks. Brain nuclei which represented different mesocortical systems were punched-out and analyzed using HPLC, and resulted as follows (p<0.05). The 50% protein diet caused elevated DA levels in the substantia nigra, the dentate gyrus, and the striatum. DOPAC:DA ratios coverted with dietary protein in tuberculum olfactorium and amygdala, but increased in the parietal cortex by the 50% and 8% diets. Both diets decreased HVA:DA ratios in frontal cortex, amygdala, striatum, interpeduncular nucleus, and were inversely related to dietary protein in the dentate nucleus. These data suggest that the nigrostriatal & mesocortical systems were more sensitive than mesocortical and mesolimbic systems to dietary protein.

634.17  
CHANGES IN STRIATAL DOPAMINE SYNTHESIS IN PREMATURELY BORN ADULT RATS AFTER INVERSIBLE RECEPTOR BLOCKADE. R. K. Catterall, C. McGuckin, G. J. Kowitt, and M. V. Bardo. Dept. of Psychology, Univ. of Kentucky, Lexington, KY 40506.  

Acute treatment with the alkylating agent N-ethylmaleimide (100 μM) in vitro reduced dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) metabolite levels and prevented increased DA levels observed in vivo following restraint stress. The present study was designed to determine if DA and DOPAC secretion in vivo following restraint stress is reduced by pretreatment with an irreversible DA receptor antagonist. Rats were pretreated with 0.01 mg/kg i.p. MK801 at this dose did not have any significant effect on DA and DOPAC levels. Twenty-four hours after MK801 treatment, all animals received an injection of radioactive [3H]DA or [3H]dopamine and sacrificed 30 min later. EEDQ treatment to protect DA receptors. Pretreatment with MK801 reduced the amplitude of a K+ -dependent outward current suggests that a common mechanism, possibly mediated by mobilization of intracellular Ca++, may be involved. Similar studies of NG108-15 cells transfected with the D2L form are currently in progress and should determine when these receptors are coupled to ion channels.  

(MH41557) (LAC), NS26081 (GRK)

634.18  
The Effect of Diclofenac on Basal and Stress-induced Dopamine Metabolism. Bret A. Morrow*, Shelly J. Rosenberg, and Robert H. Roth. Yale University, Department of Pharmacology and Psychiatry, New Haven, CT 06510.  

Restrictive selectivity activates the dopamine (DA) neurones in the medial prefrontal cortex (mPFC) and substantia nigra (SN)/ventral tegmental area (VTA) assessed by postmortem tissue measurements of DA and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) (Roth et al., Ann. N.Y. Acad. Sci. 537:138-147, 1988). This report examines the non-competitive N-methyl-D-aspartate (NMDA) antagonist, diclofenac (MK801) on the stress-induced activation of the mesocortical and mesocaudal DA systems. Rats were anaesthetized with MK801 (10 mg/kg i.p., or saline, and after a 20 min delay, were restrained or left in the home cage for 30 min. The rats were then sacrificed and the brains removed and dissected. Brain samples were homogenized, brains removed and dissected. Brain samples were homogenized, and studied in cultures which were 13-21 days old with the following procedure: cultures were grown in serum-free medium containing 10% ascorbic acid and 25 μM 5,7-dihydroxytryptamine. DA neurones were found contain three types of calcium currents. A Type current was observed which exhibited a low threshold for activation (-50 mV), displayed rapid inactivation and was partially blocked by both external application of either amlodrine or Nρ. A classic L-type calcium current was observed which could be activated from a holding potential of -40 mV, slowly and incompletely inactivated and was extremely sensitive to nifedipine. Finally, a N-type current was observed which was activated at the same threshold as the L-Type current but required prior hyperpolarization of the membrane, was transient and was blocked by external application of omega-conotoxin. DA (50-100 μM) stimulation of the D2 autoreceptors present on these cells resulted in a significant reduction in both L- and N-type calcium currents observed. Studies on the signal transduction pathways involved in mediating this autoreceptor modulation of calcium currents are currently in progress.  

(MH41557) (LAC), NS26081 (GRK)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
635.1 5-HT2 RECEPTOR LEVELS IN A7r5 CELLS ARE IRREVERSIBLY REGULATED BY RECEPTOR STIMULATION.


Toxicol. University of Southern California, School of Pharmacy. Los Angeles, CA 90033

Pyramidal cells of male castrated rats. The VTA, MFB, or NAc was observed in control animals. Chronic treatment of rats with subcutaneous morphine pellets impaired this transport by 50% (p<0.04, N=10), compared to rats which had received chronic placebo pellets.

Long-term exposure to morphine may impair the brain's endogenous reward system by altering the ability to transmit dopaminergic and other signals to neuronal elements in the NAC.

REGULATION OF SEROTONIN RECEPTORS

635.2 SIGNALLING VIA THE SEROTONIN-RECEPTOR OF C6RU-1 CELLS N. Sommermeyer and T. Glaser*. Inst. of Neuro- biology, Tropenwerke GmbH & Co.KG., Berliner Str. 156, 5000 Köln 40, Germany

The serotonin-stimulated accumulation of inositol phosphates in the rat glioma cell line C6RU-1 was characterized using a simple and rapid experimental procedure. Addition of serotonin (5-HT) to C6RU-1 cells preincubated with [3H]-inositol increased the inositol-phosphates content 2.5 to 3.5-fold with an EC50 value of 0.8 μM. The stimulatory effect of 5-HT was highly dependent on the presence of LiCl which was half-maximal effective at a concentration of 4 mM. The maximal effect of LiCl was achieved at 10 mM.

The 5-HT-stimulated Pi-response was inhibited by 5-HTT- or 5-HT1- receptor antagonists. The order of potency was spiperone = risperidone > ritanserin > pipamperone = mianserin = mepindole. Several well known neuroleptics, like cis-flupentixol, haloperidol and clozapine antagonized the 5-HT-induced Pi-response. Cis-flupentixol was about 10 times more potent than haloperidol or clozapine. The latter two exhibited their antagonistic properties only at relative high concentrations in the μM-range. Neither the 5-HT-uptake inhibitor cilazapram, the muscarinic cholinoergic antagonist phenergan, the α1-adrenoceptor antagonist prazosin, nor the β-adrenoreceptor antagonist salbutamol inhibited the 5-HT-induced Pi-response in C6RU-1 cells.

635.3 LACK OF EFFECT OF LONG-TERM ESTROGEN TREATMENT ON SEROTONIN-INDUCED OUTWARD CURRENT IN HIPPOCAMPAL PYRAMIDAL CELLS OF MALE CASTRATED RATS.


Several lines of evidence suggest an interaction between sex hormones and serotonergic neurotransmission in the central nervous system. In an electrophysiological study using current-clamp techniques Beck et al. (Neurosc., 57, 344) showed that chronic estrogen treatment (E2) for 3-4 days restored the biologic 5-HT1A-mediated hyperpolarization in CA1 pyramidal cells of ovariectomized rats. At present it is not clear whether the effects of estrogens are mediated through estrogen receptors (estrogen is aromatized to E2 in the CNS) or via a direct action of E2."
635.5 PHARMACOLOGICAL CHARACTERIZATION OF 5-HYDROXYTRYPTAMINE (5-HT3) RECEPTOR SUBTYPES IN SPRAGUE-DAWLEY RAT CORTEX, C57BL/6 MOUSE CORTEX, CD-1 (ICR) MOUSE CORTEX AND CD-1 (ICR) MOUSE ILEUM.


Membranes were prepared from sprague-dawley rat cortex, C57BL/6 mouse cortex, CD-1 (ICR) mouse cortex and CD-1 (ICR) mouse ileum. 5-HT3 receptors were labeled with [3 H](-)-(S)-N-(1-azabicyclo[2.2.2]oct-3-yl)-2,4,5,6-tetrahydro-1-H-benzo[d]isoquinolin-1-one hydrochloride mono ethanolate; [3 H]RS 42358-197 (Wong et al., Br. J. Pharmacol. 105:33P; 1992).

The radioligand bound with similar affinities (0.08 to 0.20 μM) to homogeneously population of saturable binding sites (Bmax values of 30 to 44 fmol/mg protein) in each of the tissues. However, affinities of specific agonists differed by more than 10 fold both between straining and within a single strain of mice (when comparing 5-HT3 receptor in brain cortex to those in ileum). These results demonstrate, for the first time, subtypes of 5-HT3 binding sites both between strains of mice and between tissues within a single strain of mouse.

635.7 DECREASED SEROTONIN 5-HT RECEPTORS IN THE FRONTAL CORTEX OF BRAINS SPECIFIC TO NEUROLEPTIC TREATED PATIENTS WITH CHRONIC SCHIZOPHRENIA.


Changes in serotonin 5-HT receptor number in the brains of chronic schizophrenic patients was investigated using [3 H]-LSD and [3 H]-ketanserin as ligands. The brains of 13 subjects were studied: eight who had taken neuroleptics to the death; five who had been off neuroleptics, by clinical case review, for more than one year prior to death. Ketanserin binding was saturatable with a KD of 1.3 nM and a Bmax of 195 fmol/mg protein in drug-free cases and a KD of 1.6 nM and a Bmax of 148 fmol/mg protein for drug free subjects, and a KD of 0.75 nM and a Bmax of 379 fmol/mg protein in drug-free cases and a KD of 0.75 nM and a Bmax of 162 fmol/mg protein for on drug cases (p<.05). An analysis of six matched normal controls yielded a Bmax of 153 fmol/mg protein for ketanserin (KD 1.4 nM) and Bmax of 302 fmol/mg protein for LSD (p<.02 vs. on drug cases). Neuroleptic treatment is associated with a reversible down regulation of serotonin 5-HT3 receptors in neocortical tissue from chronic schizophrenic subjects.

635.8 OVEREXPRESSION OF THE THIRD INTRACELLULAR LOOP PROTEINS OF RAT 5-HT RECEPTORS: DEVELOPMENT OF SUBTYPE-SPECIFIC ANTIBODIES AND NUCLEOPROBES.


To develop a systematic series of tools to permit the localization of 5-hydroxytryptamine (5-HT) receptors in the central nervous system, we have used the technique of polymerase chain reaction (PCR) to amplify various segments of cloned mouse 5-HT receptor cDNAs. These PCR fragments were then cloned into the expression plasmid pBAC14 and used to generate in-frame fusion proteins with the glutathione-S-transferase enzyme. These proteins were then overexpressed, purified, and used as antigens to immunize rabbits. The protein was either fixed to formaledehyde or left in its native form on the membrane. These fusion proteins can be used for electron microscopy on fixed tissues and for immunoadsorton on untiled tissue. We first applied this method to the recently sequenced 5-HT2A receptor (Albert et al., 1990). Our results show that both the "fixed" and "native" antibodies are able to immunoprecipitate CHAPS-solubilized 5-HT2A receptors labelled with [3 H]-8-OH-DPAT. In addition, both sets of antibodies recognize the same protein as our antipeptide antibodies (El Mestikawy et al., 1990), and the regional distributions are well correlated with that of the mRNA for the 5-HT2A receptor (Miquel et al., 1991). Fusion proteins from the same regions of the other G-protein coupled 5-HT receptors (5-HT1A, 5-HT1B, 5-HT1D, 5-HT3) have been obtained in our laboratory and are currently being packaged into Rabbits. Albert et al. (1990) J. Biol. Chem., 265, 5825-5832; El Mestikawy et al. (1990) Neurosci. Lett., 118, 189-192; Miquel et al. (1991) Neurochem. Int., 19, 453-465.

635.9 CHARACTERIZATION OF A FUNCTIONAL 5-HT RECEPTOR IN THE HUMAN NEUROBLASTOMA CELL LINE IMR-32.


Scatchard transformation of saturation binding to IMR-32 cells in cell membranes was investigated. Ketanserin revealed a unique, single site with a Kd of 0.57 nM and a Bmax of 147 ± 18 fmol/mg protein. Displacement studies with 5-HT3 agonists demonstrated a 5-HT3 receptor subtype that did not lower a 5-HT3 agonist (spiperone) ketanserin < mesulergine < 5-HT3). Northern blot analysis revealed two mRNA from IMR-32 cells identified two transcripts of 5.6 and 6.0kb which hybridized with a rat 5-HT3 cDNA probe. Measurements of phosphoinositide turnover during 5-HT3 stimulation was dependent increase with an EC50 = 1.9 μM and a maximal response of 3 times over basal. The effect was inhibited by spiperone (1μM) and ketanserin (1μM). Brief applications of 5-HT3 stimulation increased in [Ca2+]i in a concentration-dependent manner (EC50 = 0.5μM). These increases occurred in calcium-containing and calcium-free media, implying release of calcium from intracellular stores. Responses were blocked by ketanserin (1μM), spiperone (10μM), mesulergine (1μM) and DOI (1μM), but not by pindolol (1μM) and granisetron (1μM). 5-HT3 (10μM) hyperpolarised 34/50 IMR-32 cells (median -7mV). This response was associated with an input resistance reduction and was ketanserin/D2-sensitive. These data demonstrate a functional 5-HT3 receptor in IMR-32 cells.

635.6 ALLOSTERIC INTERACTIONS OF AGONISTS AND ANTAGONISTS AT 5-HYDROXYTRYPTAMINE (5-HT3) RECEPTORS.


5-HT3 receptors are ligand-gated ion channels and, as such, may be subject to allosteric regulatory mechanisms. To investigate the nature of ligand interactions at the 5-HT3 receptor we have examined the effects of 5-HT3 receptor agonists and antagonists on the dissociation of a selective high affinity 5-HT3 antagonist, [3H]-5-(1-azabicyclo[2.2.2]oct-3-yl)-2,4,5,6-tetrahydro-1H-benzo[d]isoquinoline-1-one hydrochloride mono ethanolate; [3 H ]RS 42358-197 (Wong et al., Br. J. Pharmacol. 105:33P; 1992), was examined.

The dissociation of [3 H ]RS 42358-197 from NG108 cell membranes was significantly slower in the presence of saturating concentrations of 5-HT3 (0.06 ± 0.01 μM) or other 5-HT antagonists than in the absence of unlabeled RS 42358-197 (0.16 ± 0.01 μM) or other 5-HT3 antagonists. One explanation for these findings is that 5-HT3 receptor agonists bind to sites distinct from the antagonist binding site to decrease [3 H ]RS 42358-197 binding by an allosteric interaction. Alternatively agonists may allosterically increase the affinity of the binding site to which the antagonist is bound thereby slowing [3 H ]RS 42358-197 dissociation. This latter interpretation is consistent with the finding that Hill slopes of competition curves for antagonists at the 5-HT3 receptor are not different from unity whereas agonists produce Hill slopes greater than 1.0. Regardless of mechanism, these findings indicate that the dissociation rate constants for [3 H ]RS 42358-197 are determined by the nature of the displacing ligand, a finding inconsistent with a competitive interaction. This, in turn, makes possible the existence of an allosteric site on the 5-HT3 receptor-gated channel towards which ligands can be directed.

635.10 HOMOLOGOUS DESENSITIZATION OF SEROTONIN-2 RECEPTORS IN RAT GLIOMA C6 BU-1 CELLS.


It has been characterized that serotonin-2 receptors are responsible for serotonin-induced intracellular calcium mobilization in rat glioma C6 BU-1 cells. As several studies suggest that serotonin-2 receptors can be desensitized and down regulated in various tissues, investigators have directed their attention to the function of these receptors. However, the precise mechanism of the desensitization of serotonin-2 receptors is so far not understood. We have now investigated the desensitization of serotonin-2 receptor-mediated intracellular calcium mobilization in C6 cells. The receptors were desensitized after pretreatment of the cells with serotonin in dose and time dependent manner. The desensitization was reversed by W-7, a calmodulin antagonist, which was co-pretreated with serotonin. Isoproterenol or thrombin did not affect the calcium response to serotonin when they were pretreated. These results suggest that serotonin-2 receptor-mediated signalling system was desensitized homologously and that the desensitization was mediated at least in part by calmodulin dependent pathway.

5-HT receptor agonists are downregulated by both 5-HT agonists (Leysen et al., 1989) but similar regulation of the 5-HT̴ receptor has yet to be studied. To explore this further, the behavioural response and 5-HT receptor protein-like immunoreactivity (5-HT̴-LI) were measured in rats and coregions following repeated treatment with the 5-HT̴ agonists (DOM) and the 5-HT̴ antagonist (m-CPP).

Adult male Wistar rats (300-335g) received twice daily injections of either DOM (2.5mg/kg ip), m-CPP (5mg/kg ip), or saline (0.154M 1ml/kg ip, 6/6 each) for 5 days. Following the first and alternate injections, rats were placed in a behavioural chamber, and a series of motor behaviours (wet-dog shakes, back, muscles contractions, rear and 90° turns) and yawning were measured separately but continuously throughout the injections. After (30min) the first injection, rats were decapitated and the 5-HT̴-LI measured by a polyclonal antiserum raised against the rat 5-HT̴ receptor protein (Sharma et al., 1992). Results were analysed by ANOVA followed by Dunnet's test (behaviour) or Student's t-test (5-HT̴-LI). Repeated injection m-CPP continued to attenuate turns and rears compared with saline (P<0.01 and P<0.001 respectively) while yawns increased progressively (P<0.05) and 5-HT̴-LI was unchanged in any brain or coregion.

A DOM-induced motor behavioural tolerance was observed confirming previously reported rapid agonist action in the rat. In contrast, no downregulation of either the hypolecotor effect (reported to be 5-HT̴ mediated, Kennett & Curzon, 1988) or 5-HT̴-LI was observed despite m-CPP administration.

636.1
IN VIVO ACTIVITY OF CP-94,253, A SELECTIVE SEROTONIN 5-HT̴ RECEPTOR AGONIST. E.D. Tingley, A.W. Schmidt, J.E. Macor, and D.W. Schatz, Pfizer Central Research, Groton, CT 06340.

Characterization of the functional properties of 5-HT receptor in vivo has been hampered by a lack of agonists having adequate selectivity for this receptor. CP-94,253, (3-[2,5,6-3H]-2,5,6-tripropyl-3,5-pyridinedione-1,4-dione) has high affinity and selectivity for 5-HT receptor (Kᵢ = 2 nM) and is 45-fold selective for 5-HT̴ vs 5-HT̴A receptors (Kᵢ, Drug Dec. Res., in press). Moreover, it causes amnesia (Koen et al., 1987) a behaviour attributed to disinhibition of 5-HT receptor (Koenet et al., Eur. J. Pharmacol., 141:429).

We have characterized the functional activity of CP-94,253 in vivo, and its effects in vivo by measuring serotonin utilization in rat and guinea pig brain. CP-94,253 behaved as a full agonist at 5-HT receptor, inhabiting forskolin-stimulated adenylate cyclase activity in a receptor-selective manner (EC₅₀: 5-HT̴A 1800 nM, 5-HT̴B 10 nM, 5-HT̴D 190 nM). One hour following ip injection, serotonin turnover in rat hypothalamus and cortex was inhibited dose-dependently (ED₅₀= 2 mg/kg) and the selective 5-HT̴ agonist 8-OH-DPAT was approximately equipotent in both species, a dose of 32 mg/kg CP-94,253 was required in order to diminish 5-HT turnover in guinea pig, a species lacking 5-HT receptor.

These data suggest that CP-94,253 acts selectively at release-modulating 5-HT receptor, rather than somatodendritic 5-HT autoreceptors.

636.3

Binding sites displaying high (5-HTD) and low (5-HTD) affinity for 5-HCarboxydopamine were revealed using [3H]-5-HT and selectively marking 5-HT receptor sites. To probe the possible heterogeneity in 5-HT receptor binding sites, we have eliminated a portion of the complex nature of 5-HT receptor by utilizing [3H]-5-HT. In corticalic regions of guinea pig brain, when using [3H]-5-HT selective labelling 5-HTD sites, 5-HT receptor competed monophasically in striatum (Kᵢ = 6,0±0.4 nm), frontal cortex (Kᵢ = 1,85±0.35 nm) and hippocampus (Kᵢ = 3,89±0.42 nm). Sumatran further differentiated amongst high affinity [3H]-5-HT receptor sites however, in both guinea pig and bovine striatum, frontal cortex and hippocampus, where biphasic displacement curves yielded a high affinity 5-HTD site, as well as a low affinity sumatran-insensitive site. Although pig brain contains a high density of 5-HTD receptors, 5-HT, 5-HT and sumatran competition for [3H]-5-HT in binding in optic tectum, brain stem, and telencephalon yielded apparently monophasic displacement curves. These results have been confirmed autoradiographically, demonstrating lack of heterogeneity in [3H]-5-HT binding in pigeon brain, while localization of multiple [3H]-5-HT binding sites in corticalic regions of guinea pig brain. Preliminary results suggest multiplicity of [3H]-5-HT binding sites in corticalic regions of human brain. These results suggest species differences exist with regard to the heterogeneity of [3H]-5-HT binding sites in vertebrate brain.

636.4

The effects of serotonin (5-HT) and selective 5-HT receptor agonists 2-m-chlorophenylindol (m-CPP) and 5-HT agonists on the dopaminergic innervation of the medial prefrontal cortex (mPFC) cells was studied using the techniques of single cell recording and microiontophoresis. The microiontophoretic application of DA (5-80 nA) produced a current-dependent suppression of mPFC cell firing. When subthreshold currents of 5-HT, 5-methyl-5-HT or 5-HT were applied, DA did not potentiate the inhibition produced by DA but not that of GABA. Similarly, the electrical stimulation of the ascending DA fiber pathway via the caudal linear raphe nucleus produced inhibition that was not potentiated by DA. The effects of potentiation were blocked by the selective 5-HT3 receptor antagonist onanitrobenson. The potentiated effects were not altered in rats pretreated with either an equimolar dose of DA or 5-HT. These data suggest that the DA actions on mPFC cells are mediated by DA receptors. However, the pretreatment with either PCPA or 5,7-dihydropyridine markedly attenuated the inhibitory action of DA on mPFC cells. Taken together, these results indicate that the inhibitory action of DA on mPFC cells depends upon 5-HT input, i.e., 5-HT may have a permissive role. Furthermore, the modulatory effect of 5-HT on DA's action is primarily mediated by 5-HT1-like receptors. The modulation of the DA effect by 5-HT1 receptor agonists may partially account for their antipsychotic potential and potential for treating drug addicts.
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The effects of 5-HT1 receptor antagonists such as ICS 205-930, MDL 72222, metoclopramide and zacopride were investigated on ethanol as well as diazepam withdrawal phenomena in the present study. There was a significant increase in locomotor activity in the ethanol as well as diazepam withdrawn rats. The treatment of rats with 5-HT1 receptor antagonists during withdrawal phase did not modify the effect in spite of the fact these agents had a slight (10-20%) depressant effect per se on locomotor activity in control rats. The ethanol-withdrawn rats were more sensitive to pentylenetetrazole (PTZ)-induced convulsions as compared to control animals. 5-HT1 receptor antagonists did not attenuate the increased sensitivity of ethanol-withdrawn rats to PTZ. Furthermore, 5-HT1 receptor antagonists did not elicit any significant effect per se on PTZ-induced convulsions in control rats. These observations indicated that 5-HT1 receptor antagonists are ineffective in attenuating hyperlocomotor-activity following abrupt termination of chronic administration of ethanol or diazepam, and increased sensitivity to PTZ in the ethanol-withdrawn rats.

636.6

5-HT1 RECEPTOR-MEDIATED ACTIVATION OF INTERNEURONS IN PERFORM CORTEX IS POTENTLY ANTAGONIZED BY RISPERIDONE, A NEW ATYPICAL ANTIPECTHIC DRUG. R.L. Gellman and G.K. Agajanian. Dept. of Pharmacology and Psychiatry, Yale University, New Haven, CT 06510.

Patent 5-HT1 receptor antagonism is hypothesized to underly the therapeutic action of new, clinically effective, atypical neuroleptic drugs such as risperidone (Lesen, et. al., 1992). Using electrophysiological techniques, we have recently shown that 5-HT1A activates, via 5-HT1A, reflexes and suppression of GABAergic interneurons located on the layer IIIIIr border of piriform cortex (Sheldon & Agajanian, 1991). Activation of these interneurons induces IPSPs in layer II pyramidal cells. In the present study we investigated the ability of several antipsychotic drugs, including risperidone, to modulate the 5-HT1A-mediated activation of interneurons and 5-HT2B, 5-HT3B, 5-HT4B in pyramidal cells.

In brain slices, 5-HT1A-activated interneurons were identified by extracellular recording using previously established criteria (Sheldon & Agajanian, 1991). The response to a bath application of 5-HT (10 μM, 1-2 minutes) was measured at baseline and in the presence of increasing concentrations of the antipsychotic drug. Risperidone dose-dependently blocked the excitatory 5-HT response with an IC50 of ~0.9 μM. Clozapine also dose-dependently blocked the 5-HT response with an IC50 of ~2 μM.

In contrast to the complete antagonism of the 5-HT response by risperidone and haloperidol, clozapine still completely blocked the excitatory 5-HT response even at the highest concentration used (10 μM). Parallel results were found for 5-HT1-activated IPSPs recorded in a rat manner and dorsal horn surgical preparations from the trigeminal ganglion. Antagonism of 5-HT1A activation of 5-HT1A receptors on a specific subpopulation of interneurons in cortical regions may be one site at which atypical antipsychotic drugs such as risperidone exert their therapeutic efforts.

636.7

SUMATRIPANT: LACK OF EFFECT ON MEMBRANE POTENTIAL OF GUINEA-PIG ISOLATED TRIGEMINAL GANGLION. H.E. Connor and C.T. O'Shaughnessy. Dept. of Neuropharmacology, Glaxo Group Research Ltd, Ware, Herts, SG12 0DP, UK. (SFPh: Brain Research Association)

The aim of this study was to investigate the effects of sumatriptan, a selective 5-HT1B/1D receptor agonist, on membrane potential of guinea-pig isolated trigeminal ganglion (TG). TGs were divided into 3 longitudinal and placed in 2-compartment baths. The d.c. potential between compartments was recorded extracellularly. Drugs were applied to the Krebs superfusion fluid of one compartment. KCl (3mM) was added to one compartment and 5-HT (0.003-0.1) to the other. A selective 5-HT1 receptor agonist, on membrane potential of guinea-pig isolated trigeminal ganglion (TG). TGs were divided into 3 longitudinal and placed in 2-compartment baths. The d.c. potential between compartments was recorded extracellularly. Drugs were applied to the Krebs superfusion fluid of one compartment. KCl (3mM) was added to one compartment and 5-HT (0.003-0.1) to the other. A selective 5-HT1 receptor agonist, 10 M to 100 M, caused small depolarisations (0.06 ± 0.02mV). Responses to each of these agents had a slight (10-20%) depressant effect per se on TG membrane potential: collagenase pretreatment of the TG with collagenase to enhance desheathing. Sumatriptan (0.1-10 μM) had no effect on TG membrane potential. These data provide no evidence to suggest that sumatriptan inhibits neurotransmission in trigeminal ganglion. Further studies are required to investigate the possibility that the anti-migraine action of sumatriptan results from a 5-HT1 receptor mediated inhibition of sensory neurotransmission in trigeminal craniovascular sensory nerves.

636.8

EFFECTS OF RENZAPRIDE AND CISAPRIDE ON FAST SYNAPTIC TRANSMISSION IN GUINEA PIG ILEUM MYENTERIC PLEXUS. H. Fan and J.J. Galligan. Dept. of Pharmacol./Toxicol., Michigan State University, E. Lansing, MI 48824

The effects of renzapride (Renz) and cisapride (Cis) on fast nicotinic excitatory postsynaptic potentials (epsps) were studied using conventional techniques in vitro. Epsps were evoked by focal stimulation of the myenteric plexus to simulate synaptic potentials and to activate interganglionic nerve strands. Drugs were applied by superfusion or by ejection from a pipette (ACH, 1 nM). Renz (n=16) at 0.03, 0.1 and 0.3 μM potentiated epsps by 22±7, 52±14, 79±21% and 85±20% respectively. The 5-HT4/5-HT3 agonist ICS 205-930 (1 μM) shifted Renz dose-response curve to the right ~30-fold. Cis alone did not affect epsp amplitude. Renz had no effect in 3 cells. Cis (n=8) at 0.1 and 1 μM potentiated epsps by 20±11, 69±25% and 88±23% respectively; this effect was blocked by ICS (1 μM). Cis had no effect in 9 cells. Renz and Cis did not affect ACH responses and resting membrane potential or resistance. These data indicate Renz and Cis can act as agonists at presynaptic 5-HT4 receptors on some myenteric neurons. Stimulation of presynaptic 5-HT4 receptors enhances ACH release. (Supported by DK 40210)

636.9


These studies were designed to test the hypothesis that glial 5-HT1 receptors mediate trophic effects of 5-HT. We found 5-HT1 receptor mRNAs and proteins expressed by embryonic day 14 raphe (RR) or substantia nigra (SN) glia in vitro. RR and SN glia conditioned media with different neurotrophic activities for 5-HT1 and TH neurons following treatment with 5-HT1 receptor agonists (10 nM 5-HT1, 8-OH-DPAT or DOI). When 5-HT or TH neurons were co-cultured with homotypic or heterotypic glia, different neurotrophic effects of 5-HT1 agonists were seen. Synthesis of NGF and IGFs in glial cultures seemed to be differentially stimulated in response to selective 5-HT1 agonists, and 5-HT and TH neurons had typical responses to 5-HT1 and IGF-1. RR and SN glia exhibited specific cAMP responses to 5-HT1 agonists, suggesting that embryonic glial 5-HT1 receptors are functional. These results indicate that during embryogenesis 5-HT1 receptors may mediate release of glial-derived factors which have neurotrophic activity for 5-HT neurons, as well as other cells in the vicinity.

636.10


A series of rotationally restricted phenolic analogs of the neurotransmitter serotonin (5HT) have been synthesized in which the 5-hydroxyindole portion of 5HT is replaced by a dihydroxyphenylalanine (Dopa, 5HT-3) or norepinephrine (NE, 5HT-4) or ephedrine (5HT-5). These compounds have very low affinity for 5HT4 receptors but high affinity for 5HT3 receptors, which are thought to play a role in the etiology of the motion sickness response (Khorana, et. al., 1983). The response to each agonist was maximally potentiated by 10-100 M each of 5HT and NE but not by antagonists of muscarinic or α1 adrenergic receptors. The response to 5HT was 2-3 fold greater than that produced by 5HT. The response to CP-132,484 was selectively antagonized by CP-132,484, but not by antagonists of muscarinic or α2 adrenergic receptors. The results indicate that a new class of compounds exemplified by CP-132,484 are 5HT3 agonists, and as such will be useful tools in the further study of 5HT receptors.

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receptor. Several standard compounds including 5-HT, spiperone and DOI itself were
agonist [125I]l-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) labelled 5-HT
affinity for the monkey rather than rat 5-HT
m onkey homogenates. As seen with the antagonist-labelled receptor, N (l) alkyl
aldehydes, without any change in its affinity constant.

of serotoninergic projections to the midbrain. Several ligands, L-tyrosine, L-tryptophan, as antagonists of post-
serotonin receptors behave as agonists at pre-synaptic sites owing to
the high affinity for serotonin versus rat agonist-labelled 5-HT receptors. The present
results confirm that the same structure-activity relationship is seen for the agonist- and
agonist-labelled 5-HT receptors. Also, the present results suggest that certain
substituted tryptamines may be useful as post-synaptic 5-HT receptors.


during the presentation. Inhibition of serotonin turnover (5 hydroxytryptophan
accumulation in NSD-1015-treated-rats) in the striatum. The high efficacy 5-HT1A
agonists, 5 15535 and 5 15931 showed > 25-fold separation. In
addition, while NAN-190 induced ptosis (reflecting n3-agonist) at doses 20-fold lower
than for 5-HT1A antagonism, S 15535 and S 15931 were active only at 25- and 7.5-fold
doses, respectively. In conclusion, S 15535 and S 15931 have high efficacy as
agonists at 5-HT1A receptors. Further, in vivo, whereas BM 7378 acted as a D2 agonist (e.g.,
inhibition of dopaminergic stereotypes) at similar doses as for 5-HT1A
agonism, 5 15535 and 5 15931 showed > 25-fold separation. In
addition, while NAN-190 induced ptosis (reflecting n3-agonist) at doses 20-fold lower
than for 5-HT1A antagonism, S 15535 and S 15931 were active only at 25- and 7.5-fold
doses, respectively. In conclusion, S 15535 and S 15931 are potent, pure and selective
agonists at post-synaptic 5-HT1A receptors. Apart from their utility as pharmacological tools, they may have the
peculiar potential as, for example, promiscuous or anxiolytics devoid of the secondary actions of
post-synaptic 5-HT1A agonists.

Stereoselective blockade of the guinea pig 5-HT
binding site by the optical isomers of

These experiments used the chiral 5-HT antagonist mettetine to examine the role of simple molecular
shape and stereochemistry at the 5-HT1D binding site and the 5-HT terminal autoreceptor in guinea pig, a proposed model of the 5-HT1D receptor activation. The 5-HT1D binding site (labelled by [3H]GTI using 1 μM 5-HT to define non-specific) and K+ stimulated [3H]-agonist release (25 mM) were measured in guinea pig frontal cortex.

These data support the identification of the terminal 5-HT
auto-receptor in guinea pig frontal cortex as a 5-HT1D receptor and reinforce similarities between the 5-HT1D and the 5-HT1B receptor, which has similar stereoelectrochemistry for the isomers of mettetine.


Preliminary experiments using molecular biology techniques suggest that there are
relatively few transmembrane changes between species variants of the 5-HT2 receptor.

The present
tryptamines, also show a similar structure-activity relationship. The novel and pure post-synaptic 5-HT1A receptor partial agonists, S 15535 and S 15931 act as autoreceptor agonists and pure post-synaptic antagonists; these properties may confer an original therapeutic profile.


This laboratory has reported that selected ergolines displayed species differences in their affinity for the antagonist-labelled 5-HT2 receptor (Nelson et al., Soc. Neurosci. Abstr. 17: Abstr. 363.15, 1991). Substitutions at the N1-position were responsible for the species selectivity seen. Since ergolines contain the tryptamine pharmacophore, the present work was undertaken to determine if simple modifications to the tryptamines, also show a similar structure-activity relationship. Selected tryptamines were therefore examined at the rat and human (5H)ketanserin-labelled 5-HT2 receptor with the following results.


Adaptive changes in 5-HT receptors were investigated in rodents after repeated administration of SR 46349B (SR). Male and female rats (230-250 g), and male and female mice (23-25 g) were treated daily for 7 days with two oral doses of 10 or 100 mg/kg/day of SR. Animals were then sacrificed and tracer studies were performed to evaluate 5-HT, receptor binding and receptor number. 

5-HT receptor number increased by 31% in female rats and by 100% in male mice. Further, 5-HT agonist administration (10 mg/kg, i.p.) produced a parallel enhancement in 5-HT receptor number, linked signal transduction and in 5-HT, receptor-mediated behavioural responses, in rodents. These findings suggest for the first time that an upregulation of 5-HT, receptors occurs following repeated treatment with a selective antagonist.
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636.17  IDENTIFICATION AND CHARACTERIZATION OF BINDING SITES FOR 3H-SETRINDOLE
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An important new advance in the pharmacological characterization of the serotonergic system has been the identification of drugs which have anti-platelet activity without extrapyramidal side effects (i.e., 5-HT agonists and antagonists) usually seen with classical neuroleptics. The exact molecular mechanism of action of these new agents is still unknown. The apparent lack of a common receptor binding profile (Sachar et al., 1991). Drug Dev. Res. 22, 239) that is correlated with the anti-platelet effects of atypical neuroleptics suggests that a yet unknown receptor mechanism may be involved. To pursue this, the binding of the tritiated form of a new 3H-selective serotonin uptake inhibitor (Skarsfeld & Lempert, Eur. J. Pharmacol. 182, 1990, 613) to rabbit brain homogenate or membrane preparations, has been studied.

3H-Seletrindole binding is trypsin sensitive with very high affinity for the serotonin transporter (Kd = 7.1 x 10^-9 M). Seletrindole binding is found throughout the brain in densities between 15 and 22 fmol/mg protein, with the highest densities found in the superficial layer of the frontal cortex. The affinity of a large number of standard compounds reveals that barbiturates, opiates (including sigma compounds), benzodiazepines, phenylalkylamines, Ca-antagonists as well as GABAergic, glutamatergic, cholinergic, histaminergic, peptidergic, and adrenergic compounds have low or no affinity for the serotonin binding sites. However, compounds with a 5-HT, and o component show high affinity for serotonin binding sites especially neuroleptics characterized as atypical.

636.18  TYPICAL TRICYCLIC ANTIDEPRESSANTS POSSESS POTENT 5-HT2 RECEPTOR ACTIVITY. B.L. Rood, HT. Meltzer and S. Craig, Department of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

For several years, we have known that many typical tricyclic antidepressants possess potent 5-HT2 antagonistic activity and produce a down-regulation of 5-HT2 receptors. Whether tricyclic antidepressants bind to other 5-hydroxytryptamine (5-HT) receptors has been relatively unexplored. With the recent cloning of several types of 5-HT receptors, we have begun to systematically re-examine the affinities and agonist-antagonist profiles of clinically-useful compounds in an effort to clarify their mechanisms of action. Using 5-HT2 receptors transiently expressed in COS-7 cells or stable cell lines, we discovered that typical tricyclic antidepressants possessed high affinities for the cloned 5-HT2 receptor. Norcyclazine, amoxapine and amitriptyline had the highest affinities (Kd's < 5 nM) while clomipramine, desipramine, imipramine, doxepin, meprobamate, and ipronidole had intermediate affinities (Kd's 20-100 nM). Ziprindine, sertraline, nomifensine, clorgyline and fluoxetine all had weak affinities (Kd's > 1000 nM) for the cloned 5-HT2 receptors. These results suggest that 5-HT2 receptor blockade could contribute to the unique action of many antidepressants (supported by the PMA Foundation).

637.1  IN VITRO BINDING PROFILE OF COMPOUNDS HAVING AFFINITY FOR BOTH DOPAMINE AND SEROTONIN RECEPTORS

In recent years efforts have been devoted to the development of non-classical neuroleptic drugs because of the several unwanted side effects of classical neuroleptics among which extrapyramidal symptoms are prominent. It appears that the severity of side effects is reduced with drugs which are also 5-HT2 antagonists. As a consequence much effort has been oriented towards obtaining drugs simultaneously affecting both systems. Within this context we have adopted a double approach investigating the pharmacological activity of compounds in which a side-replacement was combined to a functional group having affinity for the dopamine receptor and for a series of p-dimethoxybenzquinoline isosteres, where the carbon atom in position 4 was replaced by an heteroatom. The pharmacological profile of the above mentioned compounds was investigated by means of radioactive binding techniques. In particular activity on D1 and D2 receptors was evaluated using rat striatum membrane preparations. Tritiated SCH 23390 (D1) and spiroperidol (spuride displaceable, D2) were used as selective ligands. Cortical membranes and tritiated ketanserin were used to determine the activity on 5-HT2 receptors. In some occasion the activity on 5HT-1 and 5HT-3 receptors was also assayed using the appropriate ligand and tissue preparation. The binding studies indicate that p-dimethoxy-4-naphtoquinoline and the p-dimethoxy-4-naphto-thiazole compounds had the dual activity on dopaminergic and serotonergic receptors with IC50 ranging between 5.10^-10 and 1.5.10^-9 M. It appears that in this series of compounds the p-dimethoxybenzene moiety confers the affinity for both dopamine and serotonin receptors.


There has been extensive interest in central serotoninergic dysfunction as an important factor in the etiology of affective disorders. The readily accessible human platelet possesses serotonin-215-HT2) receptors and has been suggested as a possible model for the central serotonergic neuron. In this study, 5-HT-stimulated intracellular calcium(Ca) mobilization was measured in the platelets of depressed patients to assess 5-HT2 receptor function, using the Ca-sensitive fluorescent probe fura-2. Informed consent was obtained from all patients and normal subjects. The 5-HT-induced Ca response was significantly higher in unmedicated patients with bipolar disorder and melancholic major depression than in those with non-melancholic major depression and normal controls. The enhanced Ca response to 5-HT failed to correlate with severity of depressive symptoms. In patients with bipolar disorder and melancholic major depression, there was no significant difference in 5-HT-stimulated Ca response between unmedicated group and euthymic-treated group. These results suggest that 5-HT2 receptor function is increased in some type of depression and that the enhanced Ca response to 5-HT may be trait independent rather than state dependent.

637.3  ENDOGENOUS SEROTONIN UPTAKE INHIBITORS ISOLATED BY CALMODULIN-SEPHAROSE AFFINITY CHROMATOGRAPHY
J. Huchel and W. Wang. Department of Neurochemistry, Royal Ottawa Hospital, 1145 Carling, Ottawa, K1Z 7K1, Canada, and Long Beach VA Medical Center, Long Beach, CA 90822, and University of California, Irvine, Irvine, CA, 92717

In our previous attempts to isolate endogenous serotonin uptake inhibitors, we discovered endogenous compounds which were recognized by rabbit anti-imipramine antibodies. The specificity of our antisera indicated that they possess high affinity for drugs sharing a common structural component as described above. Calf brain and human plasma extracts and human urine were chromatographed on Calmodulin-Sepharose affinity column. Multiple substances which inhibit platelet serotonin uptake, 3H-imipramine binding and or "paroxetine binding were isolated and appeared to be confined to the fractions displaced by EDA. Further purification by Bio-Gel P-2 exclusion chromatography yielded subfractions which were recognized by anti-imipramine and or anti-paroxetine antibodies. The interaction of these endogenous compounds with calmodulin implicates the Ca++ dependent/calmodulin regulatory mechanism of serotonin uptake.

637.4  ANTIBODY RECOGNITION OF ENDOGENOUS SEROTONIN UPTAKE INHIBITORS
D. M. Helmstaed and W. Wang. Long Beach VA Medical Center, Long Beach, CA, 90822 and University of California, Irvine, Irvine, CA, 92717

Radioimmunoassay (RIA) enables the quantification of very low concentration of hormones or drugs in human serum or tissue extracts. In our previous attempts to isolate endogenous serotonin uptake inhibitors, we identified substances in calf brain and human plasma extracts which reacted positively with anti-imipramine antibodies (Psychiatry Research, 34:205, 1990). In order to further characterize the RIA positive substances, we characterized several polyclonal antibody preparations against other common psychotropic drugs: a neuroleptic (haloperidol), a potent non-tricyclic uptake inhibitor (paroxetine), a tricyclic antidepressant with potent norepinephrine but weaker serotonin uptake inhibition effect (desipramine) and chlorimipramine, an imipramine derivative. The affinity of these antibodies for a variety of serotonergic and related compounds as expressed in IC50 differ profoundly. Calf brain and human plasma extracts after Bio-Gel P-2 chromatography yielded fractions which differed in their antibody reactive profile. The relationship of these antibody-reactive substances to fractions which also demonstrated inhibition of serotonin uptake and "paroxetine binding is presented.
637.5

EFFECTS OF GLUTAMATE AND/OR DEXAMETHASONE ON SEROTONIN-INDUCED INTRACELLULAR CALCIUM MOBILIZATION IN C6 GLIOMA CELLS. H. Shino, M. Mikuni*, A. Kayaya, K. Kitawaki, and K. Narisawa. Dept. of Pharmacology, Univ. of Tokyo, Japan.

We investigated intracellular Ca2+ mobilization in C6 glioma cells by the addition of 5-HT4 receptor agonists. The response was inhibited by a high concentration of ketanserin (10 μM) and by the ergoline derivative
tropisetron (10 μM). Antagonists (at 10 μM) such as DOI, 5-carboxyamidotryptamine, 5-methoxytryptamine, and 5-HIAA increased cAMP in isolated ganglia included: (±)-l-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), 5-carboxyamidotryptamine, 5-methoxytryptamine, and 5-HIAA increased cAMP in neurons of the myenteric plexus of the guinea pig small intestine.

637.7

CHARACTERIZATION OF POTASSIUM CONDUCTANCE INCREASED BY SEROTONIN IN AREA CA3 OF HIPPOCAMPAL SLICES. P. Okahara, A. Ohki and S. O. Buch. Department of Pharmacology, Loyola University Chicago Stritch School of Medicine, Maywood, IL 60153.

The hippocampus receives extensive 5-hydroxytryptamine (5-HT) innervation from the median and dorsal raphe. 5-HT elicits a pronounced hyperpolarization in area CA3 hippocampal pyramidal cells through activation of a 5-HT1A receptor. The characteristics of the 5-HT1A-mediated hyperpolarization in areas CA1 and CA3 differ in many respects. This study was designed to compare the potassium conductance increased by 5-HT1A activation in areas CA1 and CA3. Standard intracellular recording techniques for current and voltage clamp were used. The hyperpolarization in area CA3 was not altered when either potassium- or calcium-EGTA phosphate electrodes were used, but was blocked with cesium chloride filled electrodes. Under voltage clamp 5-HT elicited an outward current with a reversal potential of approximately -105 mV in 3 mM KC1 artificial cerebrospinal fluid. The reversal potential shifted when the extracellular potassium concentration was changed to 5 or 10 mM KC1.

638.9


The slow depolarization evoked in enteric neurons by 5-HT is mimicked by elevation of cyclic AMP. We therefore tested the hypothesis that 5-HT increases cAMP in neurons of the myenteric plexus of the guinea pig small intestine. Ganglia were dissected from the longitudinal muscle of isolated segments of guinea pig duodenum and transverse colon and were incubated with putative agonists for 10 min at 37° C in the presence of isobutylmethyl xanthine (10 μM). This effect was three times larger than the amount of current elicited by 5-HT1A activation in area CA1. The voltage dependency of this potassium conductance is currently under investigation. These results indicate that 5-HT1A receptor activation increases potassium conductance in both CA1 and CA3. Supported by R01 NS00886 and NS24512.

638.10


The Fawn-Hooded (FH) rat strain possesses a plastid storage pool deficiency. In addition to the reduced peripheral accumulation of serotonin (5-HT), there is considerable evidence that central serotonergic function is altered in the FH rat. There is substantial behavioral and pharmacological data to indicate that 5-HT1C receptors are altered in the FH strain relative to Sprague-Dawley (SD) and Wistar rats. Earlier reports have suggested diminished [3H]lipterygic acid-3 phosphate (LTPA) and/or preincubation with saline. The present study was designed to determine if 5-HT1C receptors are altered in FH rats, but these results have not been replicated. In the study presented here, three rat strains, FH, SD and Wistar were compared for differences in both the 5-HT1C receptor and the serotonin (5-HT) uptake site. [3H]Mesulergine was used to label 5-HT1C brain receptors in four brain regions. In the hippocampus, hypothalamus, striatum and cortex there were significant differences in either the Bmax or KD values among the three strains. However, the Bmax values for [3H]esergine binding in the cortex were significantly greater in FH as compared to SD and Wistar, while affinity constants (KD) were significantly lower. [3H]Paroxetine, was used because of its selectivity in labelling the 5-HT1C site. In the cortex, the Bmax values for [3H]paroxetine binding in the cortex were significantly greater in FH as compared to SD and Wistar. Preliminary studies did not reveal any KD differences in the 5-HT1C uptake site among the three strains. The regional serotonergic differences found in the FH strain, relative to SD and Wistar, provide some support for the use of the FH rat as a genetic model for disorders such as alcohol abuse, anxiety and depression, which have been linked to serotonin dysfunction.
SEROTONIN-INDUCED POTASSIUM INCREASE IN THE RAT CELERATED CORPUS CALLOSUM FLUID AS MEASURED WITH AN 
Medical & Molecular Genetics, Indiana Univ. School of 
Medicine, Indiana, IN 46202.

Sertotonin (5-HT) exerts diverse physiological effects in the 
central and peripheral nervous systems in smooth muscle by 
interacting with pharmacologically distinct 5-HT receptor subtypes. 
The 5-HT\textsubscript{3} receptor is found in many brain regions, and 
is particularly enriched on the epithelial cells of the choroid plexus. 
To investigate whether 5-HT\textsubscript{3} receptor function may 
mediate, the level of potassium in the cerebrospinal fluid (CSF) was 
measured by an ion-selective electrode upon serotonin stimulation of 
the rat choroid plexus. Anesthetized rats were placed on a stereotaxic unit, 
and a potassium-selective electrode was positioned in the right lateral ventricle. 
Infusion of 10 \mu M serotonin solution into the left lateral ventricle consistently 
produced a small but detectable increase in the CSF potassium level. 
The increase could be detected within 1-3 min of serotonin infusion, 
and lasted between 5-10 min. These data suggest that the effect 
may be a result of the activation of the 5-HT\textsubscript{3} receptor 
on the choroid plexus upon its exposure to CSF-borne serotonin, 
which produces a modulation in the rate of potassium filtration 
across the choroid plexus.

MEDIATED CONTROL OF 5-HT NEURAL Firing IN 
GUINEA-PI G D ORAL RAPHE NUCLEUS. M.K. Mundy*, 
& Pharmacol, Q.M.C. Nottingham. NG7 2UH, U.K. I Wytech Research Ltd. 
Huntingcote Lane South, Tugby, Meltonidi, Leicestershie. SLE 6GP, U.K.

The selective 5-HT\textsubscript{1A} agonist 8-hydroxy-2-(d-aminopropyl)tetralin (8-OHDPAT) produces a 
reversal of the firing of serotonergic neurons in the dorsal 
xaph nucleus (DRN) as an effect also demonstrated in the guinea-pig DRN. Previous 
studies in the rat have demonstrated that the 5-HT\textsubscript{1A} agonist idazoxan increases the firing 
of identified serotonergic neurons in the DRN. The present study investigated 
the effects of idazoxan on 5-HT neuronal firing in the guinea-pig DRN. Male Dunkin-Harley 
guinea-pigs (280-350g) were anaesthetised with urethane (1.3g/kg i.p.) and 
the jugular vein cannulated for i.v. administration of drugs. Single barreled glass 
electrodes, filled with 2M NaCl containing 2% pumonic sky blue, were 
implanted into the DRN using stereotaxic coordinates taken from lamba (A -4.0-3.5, L 0.5, V 
-6.5-7.5mm). 5-HT neurones were identified by their slow, regular, firing pattern 
(0.5-4.0 spikes/sec) and their reversible inhibition by 8-OHDPAT (10\mu g/kg). The 5-HT 
agonist idazoxan (10\mu g/kg) significantly increased the firing rate of neurones in the 
DRN sensitive to 8-OHDPAT and revealed that these neurones exibited one of 
the two types of firing pattern. The first type consisted of a phasic bursting pattern; each 
cycle lasting between 60 and 130 secs with the rate oscillating from 4-8 spikes/sec at 
the beginning, to 20-32 spikes/sec at the end (n=7). This effect was not attenuated by 
8-OHDPAT but the 5-HT\textsubscript{1A} agonist cloidmine (10\mu g/kg) significantly decreased the 
bursting activity with normal spontaneous activity returning within 8-20 mins. With 
the second type idazoxan also increased the firing rate (92±5%, n=10) of 8-OHDPAT 
sensitive cells but without producing a phasic firing pattern. These results suggest 
that there may be two types of serotonergic cells in the guinea-pig DRN responsive to 
idazoxan. Furthermore the activity of the 5-HT neurones in the DRN of the guinea pig 
are under an 5-HT dendritic inhibitory tone as in the rat DRN.

CHARACTERIZATION OF SEROTONIN (5-HT) RECEPTORS ON NEURONS 
OF THE DIAGONAL BAND OF BROCA OF THE RAT. Wai Ling Lee* and 
J.P. Gallager. Dept. of Pharmacology & Toxicology, University of Texas 
Medical Branch, Galveston, TX 77550.

S-HT has different actions on neurons in the ventrical versus horizontal limb of the 
diagonal band of Broca (vDBB, hDBB). The principle effect of 5-HT on neurons in the vDBB was membrane depolarization (10 out of 14 neurons, 
72%); two neurons (14%) did not respond, while two were hyperpolarized. On 
the other hand, in the hDBB (n=46) the majority (70%) of neurons were 
hyperpolarized by 5-HT, 10% did not exhibit a transient or 
membrane potential change, and 10% were depolarized by 5-HT.

In an attempt to classify the sub-type of 5-HT receptor responsible for 
membrane hyperpolarization of neurons in the hDBB, agonist activity 
and antagonist efficacy were determined. 5-HT (1-30 
M) was effective, while NAN-190 (10-100 
M) was ineffective.

Twelve neurons (26.1%) had a 5-HT\textsubscript{1A} receptor subtype, 
10 neurons (21.7%) had a 5-HT\textsubscript{2} receptor subtype, 
14 neurons (30.4%) had a 5-HT\textsubscript{3} receptor subtype, 
and 8 neurons (17.3%) had a 5-HT\textsubscript{4} receptor subtype. 
Five neurons (10.9%) did not respond to 5-HT. 

Our results demonstrate the presence of different 5-HT receptors on 
neurons in the vDBB and hDBB. Furthermore, our data with agonists suggest that a different sub-
type of inhibitory 5-HT\textsubscript{k} receptor is present on hDBB neurons. 
(Supported by NSF-NSF 9246564)

SEROTONIN: PHARMACOLOGY I

BEHAVIORAL, NEUROCHEMICAL AND ANATOMICAL EFFECTS 
OF NEONATAL 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT) 
TREATMENT IN RATS. M. Mercugliano*, H.O. Nguyen, S. Djali 
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PA, 19104.

The consequences of the loss of serotonin (5-HT) neurons during early 
postnatal development were examined in rats after administration of the 
neurotoxin 5,7-DHT (100 \mu g i.v.) on postnatal day 1. Analysis of 5-HT 
content in separate groups of 4- and 8-week old rats indicated 
extensive depletion of 5-HT in the striatum and cortex, but not in the 
brainstem. Immunohistochemistry for 5-HT in 8-week old littermates 
revealed a 73% reduction in number of neurons in the dorsal 
xaph nucleus with relative sparing (27% depletion) of 5-HT neurons in the 
medial raph nucleus. Neonatal lesions of the 5-HT system were 
associated with regional changes in behavioral responses to the 
forced swimming test at 4 and 8 weeks of age. Rats treated with 
5,7-DHT showed decreased mobility during a 15-min forced swimming test as compared 
with vehicle-treated controls, which may result in an altered response to 
stress. These results suggest a regionally heterogenous effect of the 
lesea on 5-HT content, with decreases in terminal regions and 
preservation in the brainstem, in spite of significant neuronal loss in the 
spinal raph nucleus. Furthermore, lesions of the 5-HT system induced during 
the neonatal period were associated with long-term behavioral changes 
which may be mediated by the role of 5-HT in psychiatric conditions. 
Supported by USPHS grants MH 36262 and HD 25979.

Fluoxetine is an antidepressant drug that is a potent inhibitor of 5-hydroxytryptamine (5-HT) reuptake. This study describes in vivo assessment of this compound on serotonergic transmission in two brain regions. Male Sprague-Dawley rats were anaesthetized with chloral hydrate and stereotaxically injected with serotogogue probes into frontal cortex and raphe nuclei. Microdialysis samples were collected and assayed for 5-HT content. After basal levels of 5-HT were attained in both areas, fluoxetine was applied focally into one area or administered systemically as a single i.p. injection. Focal fluoxetine (100 μM) significantly increased local extracellular levels of 5-HT by approximately 400%. Both the frontal cortex and the raphe nuclei displayed similar sensitivity to fluoxetine perfusion. A concurrent decrease of 20% in 5-HT occurred in each normally perfused region following focally applied fluoxetine at the other site. Systemic fluoxetine (15 mg/kg, i.p.) also significantly increased 5-HT by about 300% in the raphe nuclei, but in contrast there was a concurrent decrease in 5-HT (50%) in frontal cortex. This is in an inverse demonstration of opposite effects of systemic fluoxetine in two brain regions. Since reuptake blockade has similar effects on 5-HT in terminal as well as somatodendritic regions, it appears that the uptake sites have similar characteristics in both areas. A decrease in 5-HT overflow in the frontal cortex following application of fluoxetine to the raphe nuclei presumably reflects the activation of somatodendritic autoreceptors. The elevation of cortical 5-HT by focally applied fluoxetine on the other hand, probably activates feedback inhibitory pathways. The net effect of systemic fluoxetine appears to be determined primarily by increased 5-HT from the somatodendritic regions which dramatically inhibits raphe neuron firing, resulting in a decrease in cortical release.

Dexfenfluramine neurotoxicity: further preclinical studies in mice and monkeys. A. Ridenour, M. Martello.

Dexfenfluramine, an anorexic amphetamine derivative, depletes central serotonin (5-HT) neurons in nonhuman primates (squirrel monkeys). Combined with similar observations in rodents (rats), these findings have raised concern that dexfenfluramine may damage 5-HT neurons in the human brain. The present study was designed to determine whether increased selective serotonin reuptake inhibitors (SSRIs) would be safely metabolized by human liver cells. Results suggest that the major metabolite of an SSRI is 5-HT, which is then further metabolized by the gut to 5-hydroxyindoleacetic acid (5-HIAA). This study demonstrates in vivo assessment of this compound on serotonergic transmission in two brain regions. Male Sprague-Dawley rats were anaesthetized with chloral hydrate and stereotaxically injected with serotogogue probes into frontal cortex and raphe nuclei. Microdialysis samples were collected and assayed for 5-HT content. After basal levels of 5-HT were attained in both areas, fluoxetine was applied focally into one area or administered systemically as a single i.p. injection. Focal fluoxetine (100 μM) significantly increased local extracellular levels of 5-HT by approximately 400%. Both the frontal cortex and the raphe nuclei displayed similar sensitivity to fluoxetine perfusion. A concurrent decrease of 20% in 5-HT occurred in each normally perfused region following focally applied fluoxetine at the other site. Systemic fluoxetine (15 mg/kg, i.p.) also significantly increased 5-HT by about 300% in the raphe nuclei, but in contrast there was a concurrent decrease in 5-HT (50%) in frontal cortex. This is in an inverse demonstration of opposite effects of systemic fluoxetine in two brain regions. Since reuptake blockade has similar effects on 5-HT in terminal as well as somatodendritic regions, it appears that the uptake sites have similar characteristics in both areas. A decrease in 5-HT overflow in the frontal cortex following application of fluoxetine to the raphe nuclei presumably reflects the activation of somatodendritic autoreceptors. The elevation of cortical 5-HT by focally applied fluoxetine on the other hand, probably activates feedback inhibitory pathways. The net effect of systemic fluoxetine appears to be determined primarily by increased 5-HT from the somatodendritic regions which dramatically inhibits raphe neuron firing, resulting in a decrease in cortical release.


The serotonergic responses to serotonin (5-HT) agonists were used to assess the state of serotonergic function after chronic treatment with antidepressants. Fluoxetine (10 mg/kg), a 5-HT uptake blocker, was administered on day 1 and continued for 10 days. The 5-HT uptake blocker, desipramine (DMI) 5 mg/kg, was given as a single daily dose for 10 days. Both were injected (i.p.) once a day for 21 days. MK-212 (5-HT1A agonist, 0.25-2 mg/kg i.p.), RU 24969 (5-HT1B agonist 0.1 mg/kg and DOI 0.1 mg/kg i.p.) was administered 18 hours after the final antidepressant injection and 30 min before decapitation. Fluoxetine potentiated the MK-212 and DOI-induced increase in plasma corticosterone, and the MK-212-induced increase of plasma ACTH level. DMI only potentiated the effect of MK-212 on plasma ACTH and corticosterone concentration. Both fluoxetine and DMI decreased food and water intake and body weight. These results suggest that fluoxetine influences 5-HT-mediated ACTH and corticosterone release, but DMI does not. Also, both fluoxetine and DMI might influence 5-HT1A and/or 5-HT1C-mediated vasopressin and oxytocin secretion. It is difficult to assess the influence of antidepressants on vasopressin secretion, because only MK-212 increased its secretion. Over time, the antidepressants maintained partial responses to 5-HT agonists. Since none of the 5-HT receptors were measured, changes were treated with fluoxetine or DMI, the influence of fluoxetine or DMI on the hormone responses to 5-HT agonists may not happen at the receptor level, but possibly at the second messenger level (Supported by MH 45812).

MK-801, a non-competitive NMDA receptor antagonist, blocks dopamine and serotonin depletions induced by fenfluramine (FEN) or FEN+MK-801. MK-801 treated rats received either 1, 2, or 4 injections of 12.5 mg/kg DL-FEN at 1 hr intervals; FEN + MK-801 treated rats received the same FEN regimens with a 2.5 mg/kg MK-801 administered 15 min before and 90 min after the first FEN injection (N=8 each gp). MK-801 did not alter FEN-induced serotonin depletions in frontal cortex and septum. MK-801 significantly enhanced serotonin depletions in striatum (1, 2, and 4 FEN injections), amygdala (1 and 4 FEN injections), somatosensory cortex (1 FEN injection), hippocampus (1 FEN injection), and hypothalamus (4 FEN injections). MK-801 alone significantly decreased serotonin depletions in striatum and amygdala. Serotonin release was measured in the striatum of awake rats using in vivo dialysis. FEN-induced serotonin release (a single injection of 12.5 mg/kg) was not altered by pretreatment (15 min) with 2.5 mg/kg MK-801.

MK-801 enhanced FEN-induced serotonin depletions in all regions except frontal cortex and septum. This result is in contrast to reports indicating that MK-801 blocks dopamine and serotonin depletions induced by methamphetamine. MK-801 did not enhance the FEN-induced serotonin release in rats indicating that the enhanced serotonin depletions with MK-801+FEN are not due to an enhanced increase in serotonin release. [Supported by NIDA DA-00085 and RSA10562 (L.S.Seiden).]


Tianeptine is considered a serotonin (4-SHT) uptake enhancer with antidepressant potential. We determined the acute neuroendocrine responses to injections of tianeptine (0-20 mg/kg, ip). Plasma prolactin was not altered by injections of tianeptine. In contrast, plasma corticosterone and renin concentrations were dose-dependent increased by tianeptine injections. The maximum increase in plasma corticosterone was observed at a dose of 10 mg/kg, 15 minutes post-injection. Plasma corticosterone returned to normal levels within 30-60 minutes post-injection. Plasma renin concentration was elevated for a longer duration. At 2 hours post-injection, plasma renin concentration was still higher than saline injected rats. To determine whether 5-HT uptake sites mediate the neuroendocrine effects of tianeptine, rats were pretreated with the 5-HT uptake blocker fluoxetine (fluoxetine: 4-SHT uptake inhibitor) 1 hour before the injection of tianeptine (2-20 mg/kg, ip). The rats were sacrificed 15 minutes after the tianeptine injection. Fluoxetine did not alter the effect of tianeptine on either plasma corticosterone or renin concentrations. The data suggest that the endocrine effects of tianeptine are mediated by a mechanism that is independent of the 5-HT uptake sites. [Supported in part by MH48512 and DA04865.]

Serotonergic projections to the hypothalamus and hippocampus influence several known functions, i.e., neuroendocrine regulation, nociception, memory and anxiety. Coronal sections of the mouse forebrain were stained with the use of a rabbit anti-serotonin antibody using the unlabeled antibody peroxidase-antiperoxidase method, including a silver postimmunization method. The stained tissue sections were projected onto white paper for cartography and semiquantitation. The regional distributions and relative density of serotonin axons for the mouse were compared with reports in the rat. The hypothalamus contained many, widely distributed axons with a density in the medial hypothalamus greater than the lateral hypothalamus. This pattern is reversed in the rat. The ventromedial hypothalamic nucleus displayed a reversed pattern compared to the rat. Other hypothalamic areas, e.g., paraventricular nucleus, contained moderate amounts of axons similar to the rat. In the hippocampus two major patterns were observed. A very high density of axons were present in the stratum lacunosum molecularae of the Ammon’s horn. The lowest density was found in the dentate gyrus. The only pyramidal cells contacted by serotonin terminals were found in CA3. This distribution is consistent with reports in the rat but varies from reports of serotonin receptor densities in the hippocampus. Supported by UTSA-Faculty Research Award 638.15


The hypothesis was tested that synaptic vesicles, which are acidic, are targets for the action of nicotine and cocaine, each of which is a weak base. Secrecion of 5-HT from synaptosomes and thyroid parafollicular (PP) cells was studied in the presence or absence of nicotine (50 μM) or cocaine (50 μM). In addition, the effects of these compounds on the actions of 5-HT on receptors in the enteric nervous system was also investigated. Cocaine, but not nicotine, increased the basal release of 3H-5-HT from synaptosomes. Both cocaine and nicotine enhanced the veratridine- and K⁺-simulated release of 3H-5-HT from synaptosomes, an effect that persisted when Ca²⁺ was lowered. Neither cocaine, nor nicotine affected the basal release of 5-HT from PF cells; however both drugs inhibited the secretion of 5-HT from PP cells in response to TSH. Trapping of acridine orange confirmed that the acidity of the 5-HT-storing PF granules increased in response to stimulation of the cells with TSH; however, both cocaine and nicotine alkalinized these granules. Intracellular records from enteric neurons indicated that nicotine (5 μM) strongly potentiated the rapid depolarization mediated by 5-HT’s receptors; this action was manifest after the depolarizing phase of the response to nicotine itself. These data show that cocaine and nicotine can interact with serotonergic elements in a complex manner that may have both pre- and postsynaptic components. Supported in part by grants NIMH 37575, NS12969, DK19743. 638.17


We examined the ultrastructural basis for known functional interactions between serotonin innervating reactive (5-HT-ir) terminals, local neurons and catecholamine afferents in the core and shell of the Acb. These monoamines were identified in the same sections of tissue using the avidin-biotin immunoperoxidase method for a rabbit SHT antiseraum and silver intensified gold labeling for a mouse antibody against the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH). By light microscopy, 5-HT-ir processes appeared less dense and topographically more heterogeneous than the TH-ir varicosities throughout the Acb. Using electron microscopy, SHT and TH-ir profiles included unmetylated and a few mymetlated axons and terminals. In the core, 5HT-ir terminals rarely formed synaptic junctions with neuronal targets but were more often found in apposition with other terminals most of which were nor TH-ir. In fact, TH and 5HT-ir axons were usually separated by a distance of >5 μm in fields containing both labels. In the shell, SHT-ir terminals were found in lower density than the lateral hypothalamus. This pattern is reversed in the rat. The ventromedial hypothalamic nucleus displayed a reversed pattern compared to the rat. Other hypothalamic areas, e.g., paraventricular nucleus, contained moderate amounts of axons similar to the rat. In the hippocampus two major patterns were observed. A very high density of axons were present in the stratum lacunosum molecularae of the Ammon’s horn. The lowest density was found in the dentate gyrus. The only pyramidal cells contacted by serotonin terminals were found in CA3. This distribution is consistent with reports in the rat but varies from reports of serotonin receptor densities in the hippocampus. Supported by UTSA-Faculty Research Award 638.15

WITHDRAWN 638.18


The hypothesis was tested that synaptic vesicles, which are acidic, are targets for the action of nicotine and cocaine, each of which is a weak base. Secrecion of 5-HT from synaptosomes and thyroid parafollicular (PF) cells was studied in the presence or absence of nicotine (50 μM) or cocaine (50 μM). In addition, the effects of these compounds on the actions of 5-HT on receptors in the enteric nervous system was also investigated. Cocaine, but not nicotine, increased the basal release of 3H-5-HT from synaptosomes. Both cocaine and nicotine enhanced the veratridine- and K⁺-stimulated release of 3H-5-HT from synaptosomes, an effect that persisted when [Ca²⁺] was lowered. Neither cocaine, nor nicotine affected the basal release of 5-HT from PF cells; however both drugs inhibited the secretion of 5-HT from PF cells in response to TSH. Trapping of acridine orange confirmed that the acidity of the 5-HT-storing PF granules increased in response to stimulation of the cells with TSH; however, both cocaine and nicotine alkalinized these granules. Intracellular records from enteric neurons indicated that nicotine (5 μM) strongly potentiated the rapid depolarization mediated by 5-HT’s receptors; this action was manifest after the depolarizing phase of the response to nicotine itself. These data show that cocaine and nicotine can interact with serotonergic elements in a complex manner that may have both pre- and postsynaptic components. Supported in part by grants NIMH 37575, NS12969, DK19743. 638.17

WITHDRAWN 638.18

Tryptophan hydroxylase (TPH) is the initial, rate-limiting enzyme in the biosynthesis of serotonin. TPH is an unstable enzyme, and detailed studies of its regulation have been limited by the lack of highly purified, active enzyme. We have utilized immunoprecipitation as a rapid, effective method to affinity purify TPH from brain. TPH was immunoprecipitated by adsorption to Pansorbin following binding to the monoclonal antihydroxylase antibody, and resuspended pellets were assayed for TPH activity. TPH could be completely immunoprecipitated from neuronal extracts in 1.5 hours, and the recovery of TPH activity using this method was 40%. Immunoprecipitated TPH (im-TPH) displayed an apparent K_m for L-tryptophan of 16 ± 13 μM, and an apparent K_m for N-acetyltyramine of 44 ± 19 μM. The V_max values for im-TPH were 33-50% of those observed for crude TPH. The thermo-stability of im-TPH was nearly identical to that of soluble TPH. Like soluble TPH, im-TPH was completely inhibited by the catechol compound apomorphine (IC_50=1.56 μM), while dopamine caused partial inhibition (54%). im-TPH was much more sensitive to activation by phosphorylation/thionine than soluble TPH. Purified calcium/calmodulin-dependent protein kinase produced a 2-fold activation of im-TPH, while purified PKA failed to activate. Using this method it was also demonstrated that PKA phosphorylates im-TPH, supporting our previous results with soluble TPH that phosphorylation of TPH by PKA does not activate the enzyme. These results infer that im-TPH retains the characteristics of the soluble enzyme and can be used to study regulation by protein kines.
369.7

**SEROTONIN (5-Ht) DECREASES INSPIRATORY-MODULATED SYNAPTIC CURRENT IN NEONATAL RAT PHRENIC MOTONEURONS. A.D. Lindsey & J.L. Feldman. Systems Neurobiology Lab., Dept. of Physiological Science, UCLA, Los Angeles, CA, 90024-1527.**

Spinal respiratory motoneurons typically respond to exogenously applied 5-HT by an increase in inspiratory-modulated firing and little or no increase in firing during expiration. The effect on呼吸的underlying mechanism was investigated. We studied the effect of 5-HT on phrenic motoneurons (PMNs) in the isolated brainstem and spinal cord preparation from neonatal rats. PMN activity was recorded intracellularly under current and voltage clamp conditions. 5-HT was applied to the phrenic nerve via pressure ejection from micropipettes in the vicinity of the PMN pool. The effect of 5-HT on exogenously applied glutamate was also studied. Local application of 1 mM 5-HT (pH 7.4) decreased peak inspiratory-modulated synaptic current (Iinsp) by 23 ± 6%. As observed by others, there was a concurrent increase in PMN excitability. This included a depolarization accompanied by a tonic inward current and increased input resistance, and a leftward shift in the f/I relationship. The 5-HT antagonist ketanserin selectively blocked the increase in excitability without blocking the decrease in Iinsp produced by 5-HT. The 5-HT antagonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (H3) mimicked the increase in excitability but did not decrease Iinsp. Under current-clamp conditions firing produced by local application of 10 mM glutamate was enhanced by 5-HT, but the underlying membrane current produced by glutamate was unaffected. Therefore, a presynaptic mechanism via 5-HT1 receptors is suggested for the 5-HT-induced decrease in Iinsp. Supported by NIH Grant NS24742 and a Muscular Dystrophy Fellowship to ADL.

369.8

**ACUTE EFFECT OF 2,4,5-TRIHYDROXYMETHAMPHETAMINE ON THE HIPPOCAMPAL SEROTONERGIC SYSTEM. M. Johnson*, H. K. Lim, R. L. Foltz, G. R. Hanson and J. W. Gibb. Dept. Pharmacology and Toxicology and Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112.**

3,4-Methylenedioxymethamphetamine (MDMA) decreases tryptophan hydroxylase (TPH) activity of the rat brain shortly after administration. TPH activity is regulated after incubation under nitrogen gas and diithiothreitol, indicating that oxidation of sulfhydryl sites on the enzyme causes the inhibition. 2,4,5-Trihydroxyamphetamine (THA) was identified as a product of MDMA metabolism and induces a long-term decrease in central tyrosine hydroxylase (TH) and TPH. The aim of this study was to determine if MDMA can produce a rapid decrease in TPH activity associated with MDMA. Male Sprague-Dawley rats (180-250 g) were injected i.c.v. with 1 μmol THM and killed 3 h later. In vivo TPH activity was measured using HPLC-EC. TH activity was determined with a radioisotopic method. THM failed to alter cortical TPH activity but reduced striatal and hippocampal TPH activity to 86% and 54% of control, respectively. Striatal TH activity remained unaffected. 6-Hydroxydopamine (1 μmol), a structural analogue of THM, failed to reduce hippocampal TPH activity but 1 μmol of 5,6-dihydroxytryptamine (5,6-DHT), a serotonergic neurotoxin, reduced TPH activity to 5% of control. This suggests that THM cyclizes to a 5,6-DHT-like compound to induce a rapid decrease in TPH activity. Since in vivo reducing conditions failed to reverse the effects of THM and 5,6-DHT, the loss in TPH activity may differ from the changes induced by MDMA. (Supported by USPHS grants DA 00869, DA 04222 and DA 05860)

369.9

**2,4,5-TRIHYDROXYAMPHETAMINE, A METABOLITE OF MDMA, DECREASES TRYPTOPHAN HYDROXYLASE ACTIVITY IN VITRO. J.M. Flayman, M. Johnson, H.K. Lim, G.R. Hanson, R.L. Foltz and J.W. Gibb. **

**Department of Pharmacology and Toxicology and Center for Human Toxicology, University of Utah, Salt Lake City, UT 84112.**

3,4-Methylenedioxymethamphetamine (MDMA) induces a rapid decline in brain tryptophan hydroxylase (TH) activity that is reversed by incubating TPH under nitrogen gas and diithiothreitol. Intracerebroventricular injection of 2,4,5-trihydroxyamphetamine (THA), a metabolite of MDMA, also induces a rapid decline in TPH activity suggesting that the metabolite may be responsible for the MDMA-induced changes. The purpose of this study was to determine if this rapid decline in TPH activity can be reproduced by THA in vitro. The hippocampus and striatum from male Sprague-Dawley rats were incubated in different concentrations of THA. Contralateral tissues were used as controls. After a 1-h incubation at 37 °C under a flow of 95% O2 and 5% CO2, hippocampal TPH activity was decreased to 3%, 47%, 68%, 71% and 83% of control after exposure to 5, 0.5, 0.1, 0.01 and 0.001 mM THA, respectively. Striatal TPH activity was reduced to 17%, 54%, 70%, 95% and 98% of control, respectively. Incubation of TPH under nitrogen gas and diithiothreitol failed to return the enzymatic activity to control levels. In contrast to MDMA, TPH activity in the hippocampus and striatum was unaffected. Therefore, a presynaptic mechanism via 5-HT1 receptors is suggested for the 5-HT-induced decrease in Iinsp. Supported by NIH Grant NS24742 and a Muscular Dystrophy Fellowship to ADL.

369.10

**IN VITRO ELECTROPHYSIOLOGIC ASSESSMENT OF AGE-RELATED SEROTONIN AUTORECEPTOR FUNCTION. H. Zheng and J.M. Laska.**

**Dept. Pharmacology and Toxicology, Univ. Texas Med. Br., Galveston, TX 77555.**

The alteration of hormonal and neuronal receptor function have emerged as an etiology common to many age-related diseases, including decline of the female reproductive axis. Aging of serotonin (5-HT) autoreceptors has been identified to include changes in cellular physiological responses mediated by a 5-HT1A autoreceptor in the dorsal raphe nucleus (DRN) as recorded in vivo in the reproducibly middle-aged and senescent rat. Using a in vitro preparation of the DRN, we have addressed the pattern of age-related decline in 5-HT receptor function in this brain region. Electrophysiologic recordings were conducted in 400μm thick slices containing the DRN from female Fischer 344 rats (6, 12, 18, 26 mol); all animals were at diestrous or constant diestrous at time of sacrifice. In slices continually perfused with 10 μM phenylephrine to activate cell firing, no significant decline in baseline spontaneous activity was observed between 12 and 26 mol groups. However, a marked age-related decline in sensitivity to 5-HT was apparent; 30 μM 5-HT produced an average of 40% vs 3% decline in cell firing in middle-aged vs old groups, respectively. Application of selective 5-HT1A antagonists NAN-190 or BMV-7378 did not attenuate 5-HT responses but, rather, produced only agonist-like inhibition of cell firing (2.5-10 μM). In summary, marked age-related decline in 5-HT receptor function is apparent in the in vitro DRN of the female rat.

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369.11

**CHANGES IN MONOAMINE LEVELS AND TURNOVER INDUCED BY SHORT-TERM FLUOXETINE TREATMENT. C.S. McKitchick*, V. Luhle, J. LePard and R.L. Stephens, Jr.*.**

**Department of Physiology, The Ohio State University, Columbus, Ohio 43210.**

Fluoxetine, a clinically active antidepressant, is believed to act by inhibiting serotonin (5-Ht) uptake in the brain, thus increasing 5-HT levels in the brain involved in affective states. Fluoxetine (10mg/kg) was administered i.p. to male rats daily for 4 days. The monoamine oxidase inhibitor, pargyline, was used to assess the effect of 5-Ht accommodation. Monoamine levels in discrete brain regions were determined by HPLC-EC; TH activity was determined with a radioisotopic method. As expected, 5-HT levels were increased 1100% and 25% in the hippocampus and striatum, respectively. However, systemic 5-HT (5 mg/kg, i.p.) inhibited pentagastrin-stimulated acid secretion by 67%. Bilateral cervical vagotomy did not reverse 5-HT-induced inhibition of acid secretion. However, systemic 5-HT (5 mg/kg, i.p.) inhibited pentagastrin-stimulated acid secretion by 67%.

**SEROTONIN (5-Ht) INHIBITS Gastric Acid Secretion by Nonulinal, Vagal Independent Mechanisms. K.J. Lepard and R.L. Stephens, Jr.*.**

**Department of Physiology, The Ohio State University, Columbus, Ohio 43210.**

Vagal stimulation increases luminal and portal 5-HT release. Previous studies suggest that released 5-HT produces an inhibitory tone on stimulated acid secretion. The mechanism of 5-HT-induced inhibition on stimulated acid secretion was investigated. In urethan-anesthetized rats with gastric and portal cannula, luminal blood 5-HT levels were measured in response to the vagal stimulant RFX7368. Basal luminal and portal 5-HT levels were elevated in ligated rats, respectively after intracisternal RFX7368. Luminal perfusion of 5-HT (10, 30, 370 ng/10 min) had no effect on pentagastrin-stimulated acid secretion. However, systemic 5-HT (5 mg/kg, i.p.) inhibited pentagastrin-stimulated acid secretion by 67%. Bilateral cervical vagotomy did not reverse 5-HT-induced inhibition of carbachol (1 mg/kg)-stimulated acid secretion. The results suggest that 5-HT acts either through luminal or splanchic afferents to produce an inhibition in stimulated acid secretion. Supported by NIH DK 42880.
639.13  GENDER AND ESTROUS CYCLE EFFECTS OF 8-OH-DPAT ON HYPOTHALAMIC SEROTONIN. S. Maswood*, O. Stewart and L. Uphouse, Department of Biology and Department of Chemistry, Texas Woman's University, Denton, Texas, 76204.

The effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) on the synthesis, utilization and turnover of 5-HT has been studied primarily in males. However, gender as well as estrous cycle differences in 8-OH-DPAT-induced behaviors have been recently reported. The present research was initiated to study the effects of 8-OH-DPAT on levels of 5-HT and 5-HIAA in female rodents and to compare the findings to males. A dose of 0.25 mg/kg of 8-OH-DPAT was injected i.p. to estrous or diestrous females and age-matched males. Thirty minutes later they were decapitated and the hypothalamus collected for HPLC analysis. Males showed the greatest change in 5-HT, 5-HIAA and the 5-HIAA/5-HT ratio. In females the effect of 8-OH-DPAT was greater in diestrous than in estrous females. These findings are consistent with previous gender and estrous cycle differences in the behavioral effects of 8-OH-DPAT.

Supported by The State of Texas Advanced Research Grant # 00346-001 and NIH RO1 HD288419


West Haven VAMC and Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Acoustic startle is a reflex with a well-characterized neural pathway that is useful as a model system to investigate effects of drugs on sensorimotor reactivity. Preclinical data indicate that the startle reflex is modulated by serotonin. Brain serotonin can be reduced by depleting plasma TRP by dietary manipulations. TRP depletion increases the startle reflex in rats. As part of a research program assessing the role of serotonin in depression, startle might provide a sensitive measure to ascertain whether TRP depletion was having central effects. METHOD: 14 depressed patients participated in two tests, one week apart. Each test involved 24 hrs. of a low TRP diet followed by a 15 amino acid drink. During one test, TRP was added to the diet and drink (control); during the other test TRP was not added (depletion). Eyeblink response to 36 tones of 5 different intensities was measured 5 hrs. after subjects received the depleting drink or the control drink. RESULTS: Plasma TRP was depleted by 70 to 90% 5 hrs. after the amino acid drink. The amplitude of the startle response was significantly increased after TRP depletion compared to control tests (p<.04). IMPLICATIONS: These findings suggest that plasma TRP depletion has central effects, and, consistent with the preclinical literature, that the startle reflex is modulated by serotonin in humans.

639.16  DIFFERENTIAL EFFECTS OF RAPHÉ GRAFTS IN THE HIPPOCAMPUS, AMYGDALA OR THE HYPOTHALAMUS. G. Richter-Levin* and M. Segal.


Serotonin depletion in rats affected spatial memory ability, body weight and thermoregulation. In an attempt to localize these functions, we studied the behavioral and physiological effects of raphe graft-induced differential restoration of the serotonin innervation of the hippocampus, amygdala and the hypothalamus, in serotonin depleted rats. Control (n=8), serotonin depleted (5,7-DHT, 200 ug, iv) (DHT, n=8), and DHT rats with raphe grafts in the hippocampus (HG, n=7), amygdala (AG, n=8), and hypothalamus (HTG, n=9), were tested in the Morris water-maze (+ atropine, 40 mg/kg, p). All lesioned groups, with the exception of the HG rats, performed significantly worse than controls. There was a significant reduction in body weight in all the lesioned groups including the HG rats. Exposure of the rats to 3 min ice cold water, led to a significantly greater reduction in body temperature in all lesioned groups, except for the HTG rats which were not different from controls. These results indicate that differences in spatial memory and thermoregulation of lesioned rats are due to its effects on body weight or thermoregulation. The graft can thus be used as a tool for studying local serotonin functions in the brain.

639.17  SEROTONIN 1A AGONIST REDUCED CONDITIONED FREEZING BEHAVIOR. T. Inoue*, T. Koyama and I. Yamashita.

Dept. of Psychiatric. and Neurol., Hokkaido Univ. Sch. of Med., Sapporo 060, Japan.

We have found that conditioned fear stress (CFS) increased serotonin (5-HT) metabolism in the medial prefrontal cortex with an induction of freezing behavior. These results could support the 5-HT hypothesis of anxiety. In the present study, the effects of various serotonergic agents and diazepam on shock-induced freezing behavior were examined using time-sampling procedure. Various doses of diazepam (0.1-5mg/kg), ipsapirone (0.1-10mg/kg), IC169,369 (5-20mg/kg), DOI (0.1-1mg/kg) or mCPP (0.1-10mg/kg) were administered subcutaneously to rats 24 hours after the last session of repeated footshock for 5 days. Rats were again placed in the shock chamber without shocks 20 min after treatment and observed. Diazepam (1mg/kg), ipsapirone (0.5-10mg/kg), DOI (0.1-1mg/kg) and mCPP (0.5-10mg/kg) significantly reduced freezing behavior. IC169,369 failed to change freezing behavior. In conclusion, these results suggest an anxiolytic potential of 5-HT1A agonist and a possible role of 5-HT1c and 5-HT2 agonist in the treatment of anxiety.
INTERACTIONS BETWEEN NEUROTRANSMITTERS IV


The contracture response mediated by α1-adrenergic receptors and the relaxation responses mediated by adenosine A2 or β2-adrenergic receptors in the isolated adventitia- and endothelium-denuded rabbit thoracic aorta were selected as a model to study functional antagonism between simultaneously activated membrane-bound receptors. These experiments focused on the effect of adenosine receptor activation on the desensitization of the response mediated by adenosine A2 or β2-adrenergic receptors. Neither adenosine nor isoproterenol altered basal tissue tension in naive tissue or in tissue preincubated with adenosine isoproterenol (10 min) had minimal effect on the concentration-response curve to phenylephrine suggesting that both receptors have fully desensitized in the absence of the α1-adrenergic receptor stimulant. Adenosine and isoproterenol rapidly relaxed phenylephrine precontracted rings in a concentration-dependent and saturable manner. The relaxation response to adenosine was monostonic and stable for over 30 min. In contrast the relaxation response to isoproterenol was biphasic, consisting of a rapid relaxation and partial recovery of tissue tension. In rings preincubated with low concentrations of phenylephrine and relaxed with adenosine, concentration-response curves to higher concentrations of phenylephrine were shifted dextrally. Taken together, these results suggest that while the adenosine A2 receptor and the β2-adrenergic receptor desensitize in the presence of their respective agonists, their desensitization is differentially attenuated by prior α1-adrenergic receptor activation: the desensitization of the adenosine A2 receptor is prevented while that of the β2-adrenergic receptor is not. USPHS GM034582.

460.3 HOMOSYNAPTIC AND HETERO SYNAPTIC GAIN MODULATION AT CO-TRANSMITTING SYNAPSES IN THE BULLFROG SYMPATHETIC VASOMOTOR C SYSTEM. R. Thom* and C.R. Hoyle Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Based on their known actions in the bullfrog, luteinizing hormone releasing hormone (LHRH) and neuropeptide Y (NPY) are capable of endowing the vasomotor sympathetic system with use-dependent synaptic gain. To test this possibility, we studied the relation between patterns of preganglionic stimuli and arterial contractions in an isolated preparation of sympathetic ganglia and the aorta (see companion paper). Short stimulus trains (≤50) evoke contractions whose amplitudes saturate in a frequency-dependent manner at 1.5 Hz. Such responses are blocked by curare and phenolamine. However, small contractions persist with longer stimulus trains. This suggests that co-transmitters can override the blockade of primary transmitters. When trains are lengthened in the absence of drugs, contractions do not saturate but continue to grow. In case, the growth in contractions was logarithmic between 2 and 4 Hz. These results suggest that co-transmitters produce homosynaptic gain modulation in this circuit.

Because the aorta is bilaterally innervated, one can test for interactions between separate pools of ganglionic ε-neurons. Using one side to evoke a test contraction and the other for conditioning, we found that conditioning trains can potentiate the amplitude of subsequent test responses. This demonstrates that interactions occur between postganglionic synapses and is most simply described in terms of enhanced heterosynaptic gain.

Supported by NIH grants NS21065, NS01427 and HD07343.


We studied the possible neurotransmitter involved in the modulation of GABA release from granule cells of the olfactory bulb. The GABergic granule cell has been well characterized, however the neurotransmitter of the mitral cell, which establishes reciprocal synaptic coupling with the granule cell, is controversial. A strong candidate is glutamate. In previous work (Jaffé, Veleo 1989, J. Neurochem. 52,1764-1776) we observed that the GABA release response on the release of GABA and was only seen after a previous K depolarization. This effect of Glutamate was indirect since it was inhibited by Mg2+ and D-APV. We further characterized the effect using continuous superfusion of olfactory bulb slices prelabeled with 3H-GABA. Nippecotic acid a GABA uptake inhibitor enhanced the GABA releasing effect of Glutamate, Kainate and AMPA. The effect of NMDA was only seen after a previous depolarization of the tissue with K or Kainate. The effect of AMPA and NMDA was inhibited by CNQX but not by AP5. TTX inhibited Glu and Kainate effect. The excitatory amino acid, cysteinesulphonic acid, was able to induce GABA release. At the level of the olfactory bulb, excitatory amino acids are able to induce GABA release through a polysynaptic mechanism.

460.6 THE EFFECT OF CHRONIC HALOPERIDOL ADMINISTRATION ON GABA-IMMUNOREACTIVE AXON TERMINALS IN RAT MEDIAL PREFRONTAL CORTEX. E. Adate*, A. Vincent, I. Sternson and F.M. Beneh. Department of Anesthesia, Massachusetts General Hospital, Boston, MA; Department of Psychology and Program in Neuroscience, Harvard Medical School; Maimon Research Center, McLean Hospital, Belmont, MA.

Several reports provide evidence that chronic haloperidol treatment induces ultrastructural changes in synapses in substantia nigra, corpus striatum and medial prefrontal cortex (mPFC). Recent studies suggesting that there is a loss of GABAergic cells in anterior cingulate cortex of schizophrenic subjects have prompted interest in the question of dopamine blocking agents can influence this transmitter system. This study provides a quantitative light microscopy analysis of GABA-immunostained axosomatic terminals in mPFC of rats treated with haloperidol. The results show that GABA-immunostained axosomatic terminals were used: cultures containing predominantly neurons and mixed, containing neurons and glia. Both types were able to incorporate radioactive GABA into a matrix dependent on temperature and pH of the medium but independent on the presence of extracellular Na. Nipperic acid, a potent inhibitor of GABA release, did not interfere with the uptake of (H)p-putrescine, suggesting that the GABA release of putrescine is distinct from the mechanism of GABA uptake. In mixed cultures containing predominantly neurons, both GABA-15% and toluene 100M released radioactivity, the effects also being Ca-dependent. Glutamate, a compound that releases GABA in the retina, did not increase the release of radioactivity in a calcium dependent manner. In cultures containing predominantly neurons, both GABA uptake and release, the radioactive material was taken up by the GABA-immunostained axosomatic terminals. The results suggest that GABA plays a role in the regulation of GABA release and that its effects on GABA release are not blocked by GABA uptake inhibitors. Our results suggest that, although participation in the synthesis of GABA, may have a neuromodulatory role in the retina. Supported by: CNPq - FAPEPA - Pront-UFF.

460.7 SYMPATHETIC INNERVATION OF THE BULLFROG AORTA BY 2 CO-TRANSMITTING SYNAPSES IN SERIES. J.R. Kline, W. Stover and R Thorne Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

We seek to interpret the integrated physiological responses of the sympathetic nervous system when two GABAergic neurons innervate an organ. The bullfrog sympathetic vasomotor c system is a suitable preparation for such a study. We have described a preparation made from a bilateral dissection of paravertebral ganglia 7-10 and the abdominal aorta. It has 2 co-transmitting synapses in series and is suitable for observing the transformation between controlled patterns of preganglionic stimulation and evoked aortic contractions. Anatomically, the lumbar ganglia innervate the aorta. In addition to the release of GABA, the sympathetic nervous system innervates the aorta. Nerve-evoked contractions can be recorded for many hours after amputating a 1 cm length of the aorta to a tension transducer. Stimulation of the preganglionic C, but not the B, pathway causes contraction. The aorta is bilaterally innervated. Nerve cuts show that ganglia 9 & 10 provide at least 90% of the innervation to the caudal aorta. Blockers of nicotinic and α-adrenergic receptors antagonize the evoked aortic contractions. Evidence for synaptic gain is presented in the companion paper. Supported by NIH grants NS21065, NS01427 and HD07343.


Polysamines are a group of homologous molecules that may act as second messengers or as transcription factors. They are also able to inhibit the uptake of (3H)-putrescine, suggesting that the ability to incorporate radioactivity in neuronal cultures, suggesting that the release of radioactivity in a calcium dependent manner. In cultures containing predominantly neurons, both GABA uptake and release, the radioactive material was taken up by the GABA-immunostained axosomatic terminals. The results suggest that GABA plays a role in the regulation of GABA release and that its effects on GABA release are not blocked by GABA uptake inhibitors. Our results suggest that, although participation in the synthesis of GABA, may have a neuromodulatory role in the retina. Supported by: CNPq - FAPEPA - Pront-UFF.
640.7 E ffects of typical and atypical antipsychotic drugs on extracellular GABA levels in the pr efrontal cortex. D. Cameron, A. J. Bourdelais, and A. Y. Dreutch. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508, and VA Medical Center, West Haven, CT 06516.

Dopamine (DA) afferents to the prefrontal cortex (PFC) inhibit cortical pyramidal neurons through both direct and indirect means. Direct synaptic connections between DA terminals and pyramidal neurons are not present. DA indirectly inhibits pyramidal cell activity by enhancing GABA release from interneurons. We have used in vivo microdialysis to characterize the effects of typical and atypical antipsychotic drugs (APDs) on extracellular GABA levels in the PFC. Animals were implanted with chronic indwelling guide cannulas, and one week later a dialysis probe place d in the area. The freely-moving rate was then perfused with dialysis buffer until a stable baseline was obtained, and then either the typical APD haloperidol or the atypical APD clozapine was injected subcutaneously. Haloperidol resulted in a marked decrease in extracellular GABA levels as compared to vehicle control. In contrast, clozapine did not significantly reduce extracellular GABA levels in the PFC compared to its vehicle. Pre- and post-treatment with clozapine both significantly reduced extracellular GABA levels whereas pretreatment with haloperidol did not affect the baseline release. Previous results have shown that clozapine results in a greater enhancement of DA release in the PFC than does haloperidol, consistent with the lower affinity of clozapine for the D2 receptor. Clozapine may not block postsynaptic activity of DA as much as haloperidol. The present data suggest that the atypical APD clozapine may act on negative symptoms by not enhancing GABA-induced inhibition over cortical pyramidal neurons. Supported by MH-45124 and VA National Center for Schizophrenia Research and Post Traumatic Stress Disorders at the West Haven VA Medical Center.

640.8 E ffects of typical and atypical antipsychotic drugs on glutamic acid decarboxylase gene expression. P.Z. Gallippo* and A. Y. Dreutch. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508 and VA Medical Center, West Haven, CT 06516.

Dopamine (DA) appears to regulate the activity of GABAergic neurons in both the striatum (CP) and in the prefrontal cortex (PFC). We have therefore examined the regulation of glutamic acid decarboxylase (GAD) gene expression in the PFC following chronic (21 day) administration of antipsychotic drugs (APDs); these include the typical APDs haloperidol and remoxipride, the atypical APDs clozapine and raclopride, and the putative atypical APD remoxipride. Preliminary data suggest that haloperidol and raclopride increased expression of both GAD3 and GAD67; remoxipride only marginally increased GAD67 expression; but apparently did not increase GAD65 expression. The effects of haloperidol and raclopride on GAD mRNA levels appeared to be more pronounced on GAD65 mRNA. These data suggest that APDs increase GAD gene expression in the striatum, but that there may be differences between two typical APDs (haloperidol and raclopride) and remoxipride, a putative atypical APD. Supported by MH-45124 and VA National Center for Schizophrenia Research to the West Haven, CT VA Medical Center.
640.14 

**EFFECT OF 6-HYDROXYDOPAMINE (6-OHDA) LESIONS IN NEONATE RATS ON mRNA LEVELS ENCODING THE ENZYME GLUTAMATE-DECARBOXYLASE (GAD) AND THE DOPAMINE D2 RECEPTOR.** J. Schopenhauer, Centre de Rech. en Neurobiologie, Univ. Laval, Quebec, CAN. 

6-OHDA lesions of dopamine neurons in adult rats increase mRNA levels encoding the dopamine D2 receptor and GAD enzyme in projection neurons of the striatum. When dopamine neurons are lesioned in neonates, adults exhibit specific behavioral and neurochemical features not observed after adult lesions. In this study, we determined if 6-OHDA lesions (intraventricular injections in neonate Sprague-Dawley rats) induce changes in mRNA levels encoding the enzyme GAD (Mns 67,000) and the dopamine D2 receptor at adulthood. Brain sections were processed for in situ hybridization histochemistry with 35S-labeled probes. Labeling was visualized by X-ray film autoradiography and measured by computerized densitometry. In agreement with previous studies, we found increased and decreased levels of mRNAs encoding the enzymes enkephalin and substance P, respectively, in sections of the striatum. Adjacent sections of 6-OHDA-treated rats exhibited an increased labeling when processed with the dopamine D2 receptor probe (+114% as compared to controls) or with the GAD (Mns 67,000) probe (+119% and +135% as compared to controls for stereotaxic levels IA 10 and 10.5, respectively). Both D2 and GAD mRNA increases appeared homogeneously distributed in the dorsal-ventral and lateral-medial portions of the striatum. The results indicate that injection of 6-OHDA in neonate rodents can modify the level of expression of GAD and dopamine D2 receptor mRNAs in a direction similar to that observed after adult lesions. (Supported by FRSQ)

640.13 


Stress activates the mesocorticolimbic dopaminergic (DA) neurons (Thiry et al., Nature 329:547, 1987). The present study sought to determine whether the stress-related peptide, adrenocorticotropin (ACTH), makes synaptic contacts with mesocorticolimbic DA neurons. Single- and double-labeling immunocytochemical staining procedures were used to examine the relationship between ACTH containing nerve terminals, and DA neurons in the rat midbrain. ACTH nerve terminals were found extensively in regions occupied by the mesocorticolimbic DA neurons, such as the interfascicular, paranigral and central linear nuclei. CLi (in the CLi, ACTH axon terminals made both symmetric and asymmetric synaptic contacts with DA dendrites, and putative axo-axonic contacts with unlabeled axon terminals which, in turn, made contacts with DA dendrites. The ACTH-DA synapses may play a role in stress-induced changes in mesocorticolimbic DA neuronal activity. Supported by grant DA-05314.

**BEHAVIORAL PHARMACOLOGY:** 

**DOPAMINE, SEROTONIN, NE**

641.1 


Recent evidence suggests that the behavioral effects of high- and limited-ef
cacy D1 agonists may differ in primates. In the present study, the unconditioned behavioral effects of the high-efficacy D1 agonists SKF 81297 and SKF 82958 (0.03 - 1.0 mg/kg) and RSKF 38393 and SKF 75670, and the D2 agonists (+)-PHNO and quinpirole were compared by videotaping unconditioned behavior in squirrel monkeys in their home cages following the administration of drug or vehicle. Videotapes were scored by at least 2 observers for different behaviors using a continuous observation procedure. The high-efficacy D1 agonists SKF 81297 (0.1 - 3.0 mg/kg) and SKF 82958 (0.03 - 1.0 mg/kg) produced dose-dependent increases in the frequency of visual scanning and had no effect on either huddling or scratching. The limited-efficacy D1 agonists RSKF 38393 (0.1 - 1.0 mg/kg) and SKF 75670 (0.1 - 1.0 mg/kg) produced dose-dependent increases in the frequency of huddling and had no effect on visual scanning or scratching. The D2 agonists (+)-PHNO (0.0003 - 0.01 mg/kg) and quinpirole (0.003 - 0.3 mg/kg) produced dose-dependent increases in the frequency of scratching and had no effect on visual scanning or huddling. The present results indicate that the unconditioned behavioral effects of high- and limited-efficacy D1 agonists differ in primates and that the unconditioned behavioral effects of D2 agonists differ from those of D1 agonists. Supported by USPHS Grants DA37743, DA04993, MH07658, and RR01068.

641.3 

**Blockade of S2 receptors in rats enhances D1-mediated repetitive jaw movements.** R.U. Schweitzer and A.J. Friedhoff, Millhauer Laboratories NYU School of Medicine Department of Psychiatry, New York, NY 10016. 

We have previously demonstrated that the D1 dopamine system mediates behavior we have named [RJ] repetitive jaw movements in rats. This behavior can be induced by the D1 agonist SKF 38393 and inhibited by the D2 agonist, L717,555, or enhancement of the antagonism of dopamine receptors by substance P. The role of dopamine receptors in this behavior has been confirmed by the injection of intracerebroventricular, intraventricular, or intracerebral injections of dopamine receptors. We have shown previously that inactivation or blockade of striatal D2 serotonin receptors with cyproheptadine on this behavior. We found that inactivation or blockade of S2 receptors greatly augmented SKF 38393 - inducible RJM. The present findings demonstrate that the S1 system inhibits D1-mediated RJM. These findings provide a mechanism underlying the clinical use of serotonin-2 antagonists in the treatment of tardive dyskinesia.

641.2 

**Intra-striatal injections of kainic acid induce contralateral rotation in rats.** L.D. Smith and R.J. Beninger, Queen's University, Kingston, Ontario, Canada. K7L 3N6. 

The role of striatal kainate receptors in the control of locomotor activity was investigated with intra-caudate injections of kainic acid (KA). Kainate was injected in three concentrations (5 μM, 250 μM, 250 μM dissolved in 0.5 μM saline) into the dorsal striatum of 15 rats. A significant increase in contralateral turning was observed after 250 μM (p<.05) and 50 μM KA (p<.05) whereas the 5 μM dose had no effect. In addition, 250 μM KA resulted in an increase in the number of rotations exhibited during the observation period (p<.05). Behavioral evidence of seizure activity was not observed at any dose level.

Excitatory corticostriatal projections have been shown to depolarize striatal output neurons via a non-NMDA glutamate receptor subtype. Thus a KA receptor-mediated increase in striatal cell firing may underlie the activation of motor systems, resulting in rotation. Striatal glutamate receptor activation also stimulates the release of neurotransmitters such as dopamine (DA). Since rats with imbalances in striatal DA receptor stimulation tend to rotate away from the side of higher DA, it is possible that the contralateral turning resulted also from a KA-induced increase in extracellular DA levels. Additional experiments using systemic KA injections will examine the involvement of an interaction between glutamate and dopamine receptor stimulation in the observed behavior. (Supported by NSERC)

641.4 


Typical stereotypes (nibbling-licking-biting) and climbing in normal rodents can be induced only by stimulation of both D1 and D2 receptors (Braun and Chau, 1986; Moore and Axton, 1988). However, either D1 or D2 agonists can reverse reserpine-induced akinesia, as measured by an increase in locomotor activity, in mice (Rubinstein et al., 1988). Thus, the effect of dopamine agonists and antagonists on stereotypes (S) and climbing (C) in mice with supersensitive receptors (produced by 20-24 hour pretreatment with 5.0 mg/kg sc reserpine) can be inferred from this model. The mixed D1/D2 agonist, apomorphine, dose-dependently increased S and C in normal mice (S-ED50=2.5 and C-ED50=0.09 mg/kg sc). Quinpirole, the full D-2 agonist, significantly increased S and C (S-ED50=0.2 and C-ED50=0.09 mg/kg sc). The mixed D1/D2 weak partial agonist/antagonist, 38393 (10.0 mg/kg ip), induced increase in extracellular DA levels. Additional experiments using systemic DA agonist injections will examine the involvement of an interaction between glutamate and dopamine receptor stimulation in the observed behavior. (Supported by NSERC)

641.5 

**EFFECTS OF 6-HYDROXYDOPAMINE (6-OHDA) LESIONS IN NEONATE RATS ON mRNA LEVELS ENCODING THE ENZYME GLUTAMATE-DECARBOXYLASE (GAD) AND THE DOPAMINE D2 RECEPTOR.** J. Schopenhauer, C. Do, and D. German, The Neuroendocrine Unit, Univ. of Rochester Med. Sch., Rochester, N.Y. 14622. 

6-OHDA lesions of dopamine neurons in adult rats increase mRNA levels encoding the dopamine D2 receptor and GAD enzyme in projection neurons of the striatum. When dopamine neurons are lesioned in neonates, adults exhibit specific behavioral and neurochemical features not observed after adult lesions. In this study, we determined if 6-OHDA lesions (intraventricular injections in neonate Sprague-Dawley rats) induce changes in mRNA levels encoding the enzyme GAD (Mns 67,000) and the dopamine D2 receptor at adulthood. Brain sections were processed for in situ hybridization histochemistry with 35S-labeled probes. Labeling was visualized by X-ray film autoradiography and measured by computerized densitometry. In agreement with previous studies, we found increased and decreased levels of mRNAs encoding the enzymes enkephalin and substance P, respectively, in sections of the striatum. Adjacent sections of 6-OHDA-treated rats exhibited an increased labeling when processed with the dopamine D2 receptor probe (+114% as compared to controls) or with the GAD (Mns 67,000) probe (+119% and +135% as compared to controls for stereotaxic levels IA 10 and 10.5, respectively). Both D2 and GAD mRNA increases appeared homogeneously distributed in the dorsal-ventral and lateral-medial portions of the striatum. The results indicate that injection of 6-OHDA in neonate rodents can modify the level of expression of GAD and dopamine D2 receptor mRNAs in a direction similar to that observed after adult lesions. (Supported by FRSQ)
LOCOMOTOR ACTIVITY IN CONTROL AND CHRONIC
CAFFEINE-TREATED MICE: INTERACTIONS OF ADENOSINE
ANALOGS, CHOLINERGIC AND DOPAMINERGIC AGENTS
AND XANTHINES. O. Nikodemie, K. A. Jacobson, and J. W.
Daily, NIDDKNIH, Bethesda MD 20892.

Chronic caffeine ingestion (CCI) by NIH Swiss male mice results in a prolonged reduction in locomotor activity and alterations in response to caffeine, other xanthines, adenosine analogs, and nicotinic and muscarinic agents. Caffeine (IP) and an A2-selective xanthine (3,7-dimethyl-1-propargylxanthine) remain stimulatory, and the typical bell-shaped locomotor dose-response curve to caffeine is left-shifted after CCI. Mice became more sensitive to depressant effects of A1 and A2 agonists. Depressant effects of xanethines that are potent PDE inhibitors are either blunted or enhanced by CCI. Depressant effects of nicotinic agonists are either little affected (oxotremorine, agonist) or right-shifted (scopolamine, antagonist). Depressant effects of the mixed A1/A2 agonist NECA, either alone or in combination with caffeine or other xanethines, are altered after CCI. Depressant effects of NECA in the presence of scopolamine or amphetamine, are similar in controls and after CCI. The depressant effects of a low dose of NECA in the presence of cocaine are reduced after CCI. The results suggest complex effects of CCI on adenosine-, dopaminergic-, and cholinergic-mediated behaviors.

THE NUCLEUS ACCUMBENS AND CAUDATE AS NEURAL MEDIATORS OF AMPHETAMINE-INDUCED LOCOMOTOR STEROYLETY. P. E. Krueger and R. Magill, Division of Psychology, Texas Christian University, Fort Worth, TX 76129.

Amphetamine (AP) produces stereotypy in rats that include focused stereotypy, hyper-locomotion, and locomotor stereotypy (patterened locomotion). The mechanisms controlling hyper-locomotion (nucleus accumbens [NAC]) and focused stereotypy (anterior caudate/CAUD) are well understood, but how neuroleptics affect these is not well understood. Two experiments examined the NAC and CAUD for the production of locomotor stereotypy as assessed by the gymnastik procedure. In experiment 1, fluoxetine (FLU) (0.0, 1.25, 2.50 mg/kg) was infused either into the NAC or CAUD prior to systemic amphetamine (2 mg/kg). In the NAC, both doses of FLU reduced hyper-locomotion and locomotor stereotypy. In the CAUD, neither dose affected hyper-locomotion, but the lower FLU dose was more effective than the higher dose at reducing locomotor stereotypy. In experiment 2, 100 mg/kg amphetamine was infused into either the NAC or CAUD. In the NAC, amphetamine produced hyper-locomotion but did not produce locomotor stereotypy. Amphetamine in the CAUD produced focused stereotypy without producing locomotor stereotypy. These results suggest the involvement of both the NAC and CAUD in the production of amphetamine-induced locomotor stereotypy.

CHOLINERGIC, GLUTAMATERGIC AND OPIOID INDUCTION OF DOPAMINE (DA) RELEASE: IMPACT ON THE STIMULUS PROPERTIES OF D1 AND D2 DA AGONISTS. R.A. Fox, D.G. Mancinelli, and B.D. Davis, Environ, Health Sci. Ctr. and Interdepartmental Neuroscience Program, Univ. of Miss., University, MS 38677.

It has become increasingly clear in recent years that neurotransmitter systems do not operate in isolation, but are interactive, regulating aspects of each other's function. Non-competitive NMDA receptor antagonists, mu opioid agonists and muscarinic cholinergic agonists all have been reported to increase DA release, as measured by in vivo microdialysis. To determine whether this DA release has functional or behavioral properties, the ability of these compounds to substitute for DA agonists in a drug discrimination (DD) paradigm was examined. Rats were trained to discriminate either the D1 agonist SKF89393 (6.0 mg/kg) or the D2-type agonist quinpirole (0.05 mg/kg) from saline using a standard two-lever, food-reinforced DD paradigm. Following acquisition of the discriminations, the ability of the non-competitive NMDA receptor antagonist AP5 (10, 20, 40 mg/kg) to substitute for the D1 agonist was examined. As the NMDA antagonist AP5 was increased from 0 to 20 mg/kg, there was a trend toward a decrease in DA release (p < 0.05). Depletion of DA in the NAcc by 70% did not alter the ability of AP5 to substitute for the D1 agonist. These data suggest that the behavioral properties of compounds that act directly on glutamatergic, opioid or cholinergic neurotransmitter systems do not necessarily operate in isolation, but are interactive, regulating aspects of each other's function.

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441.11

DOPAMINE D1 AND D2 MEDIATION OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF TRADIMELON. S.-K. Pierre,* and D.A. Eckenrode. Dept. Of Psychology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Tridimelon (FR), a triphenylmethyl compound, has been shown to increase motor activity and to induce stereotypy. Behavioral effects that are characteristic of the psycho-motor stimulants. Tridimelone was also found to function as a discriminative stimulus, and its stimulus properties were found to be qualitatively similar to those produced by the psycho-motor stimulants, i.e., amphetamine.

A variety of evidence suggests that dopamine (DA) may be important in mediating the behavioral effects of TDF. The purpose of the present study was to assess the role of DA in the discriminative stimulus effects of TDF. The primary dependent variable was nose poking at doses of up to 4 mg/kg did not substitute for the TDF stimulus. These preliminary data suggest a role for the involvement of the D2 receptor in the discriminative stimulus properties of TDF.

441.12


Two experiments were conducted to elucidate the variables that control the development of sensitization to the hypophagic effects of haloperidol (HAL). In the first experiment, groups of rats were given chronic intra-oral injections of either HAL (0.62 mg/kg) or saline at interdose intervals (IDs) of 1, 2, 7, or 14 d.

Following each injection, the rats were permitted access to food for a period of 30 min. HAL given at IDs of 1 or 2 produced neither tolerance nor sensitization to the initial hypophagic effect of the drug, whereas at IDs of 7 or 14 d, sensitization developed. In the second experiment, groups of rats were given injections of HAL (0.31 mg/kg) at 7-day intervals either before (Before Group) or after (After Group) access to sweetened milk. Control groups were given injections of saline prior to milk access.

After sensitization developed in the Before Group, all groups received a single injection of HAL (0.15 mg/kg) before access to milk. On this final test, only the Before Group showed sensitization of hypophagia. The After Group ingested as much as the control groups. These results demonstrate that sensitization to HAL-induced hypophagia develops at IDs greater than 2d and is contingent on access to milk while in the drug-free state. (Supported in part by grant DA-04592 from NIDA)

441.13

REINFORCING EFFECTS OF THE D1 DOPAMINE AGONIST SKF 12197 IN RHESUS MONKEYS. M.R. Weed, K.F. Vanover and J.L. Woolverton.* Drug Abuse Research Center, The University of Chicago, Chicago, IL 60637.

The partial D1 agonist SKF 38393 has previously been found not to function as a positive reinforcer in rhesus monkeys (Woolverton et al., JPET 230:678, 1984). The present experiment was designed to evaluate the reinforcing effects of the full D1 agonist SKF 12197 under similar conditions. Sucrose solutions were prepared in chronic intravenous catheters and lever pressing was maintained by cocaine (0.13 mg/kg, FR10), saline (0 mg/kg) under baseline conditions. When responding was stable (+/−10% of 3 day mean), saline was substituted for cocaine. Responding decreased to low levels (<10% FR10) within 4−6 sessions. Doses of SKF 12197 (SKF: 0.003−0.3 mg/kg) were then made available for at least 4-6 sessions or until responding was stable above baseline conditions. Baseline conditions were reinstated between SKF doses. Responding was maintained above saline levels at least at two doses in both monkeys. Injections of the D2 antagonist SCH 39166 (SCH: 0.003−0.1 mg/kg, i.m. 30 min pre-session) were administered before alternate sessions over the last several sessions of availability of the lowest SKF dose self-administered (0.01 mg/kg). SCH decreased responding maintained by SKF in a dose-related manner in both monkeys. At the highest SCH doses, rate and pattern of responding were similar to that seen when saline was available for self-administration. Thus, the D1 agonist SKF functioned as a positive reinforcer and its reinforcing effect appeared to be blocked by the D2 antagonist SCH. These results further implicate D1 receptors in the reinforcing effects of drugs which increase dopamine neurotransmission. (Supported by NIDA grants DA-00250, DA-00161 and NIGMS grant GM-07151).

441.14


Iodoxazol is the most selective D2-adrenergic antagonist to date which produces an interoceptive discriminative stimulus or "cue" in rat drug discrimination (DD) studies (Sanger et al., 1989, Psychopharmac. 99, 117-121). However, this drug also bind with high affinity to the non-adrenergic idazoxan binding site (NAIBS, Michel & Insel,1989, Trends Pharmacol. Sci. 10, 342-344). The 2-ethoxy analogue of idoxazol, RX811059 is a highly selective D2-receptor antagonist with minimal affinity to NAIBS (Malila et al. 1991, Br. J. Pharmacol. 102, 221P).

The purpose of this study was to examine the ability of RX811059 to produce a discriminable cue in rats. A group of male 20-day-old hooded rats (n = 6) learned to discriminate RX811059 (2.5 mg/kg, ip) from saline in a fixed ratio (FR = 10) DD schedule. A series of D2-receptor antagonists were tested for their ability to generalise to (i.e. mimic) the RX811059-induced cue. RX811059 itself dose-dependently mimicked the RX811059-induced cue (i.e. >80% total responses were to RX811059 associated) as did clonidine, an adrenergic receptor agonist, using doses up to 0.8 and 0.4 mg/kg respectively. However, the peripherally acting adrenoceptor antagonist idazoxan, showed no effect when given at doses > 3 mg/kg. These results demonstrate that RX811059 functions as a D2-adrenoceptor antagonist and does not bind with high affinity to the NAIBS. (Supported in part by grant DA 04592 from NIDA, NIDA, the National Institute on Drug Abuse, Department of Health and Human Services, Washington, D.C.)

441.15

THE INVOLVEMENT OF A2-ADRENERGIC MECHANISMS IN STRESS-RELATED BEHAVIORAL CHANGES. A. Garmann and Adrijan J. Dunt*, Dept of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

Substantial evidence suggests that noradrenergic systems are involved in stress-related behavioral responses. Previous studies showed that a2-adrenergic agonists induce conditioned 200 Hz ultrasonic vocalizations (USVs) when administered intraperitoneally or in the absence of further stimulation (Yan et al., 1990). These USVs were increased by repeated presentation of a CS+ which increased 200 Hz vocalizations in mice tested in the multicompartement chamber (McC Beggins & Dunn, 1988). Because the a2 agonist-induced behaviors were attenuated by blocking the 5-HT1A receptor, the conclusion was drawn that a2 neuroadrenergic systems may be responsible for these anxiety-like responses. L-Propranolol (2.5-5 mg/kg) decreased defensive withdrawal in naive rats and reversed that induced by restraint or i.c.v. CRF (Yang et al., 1990).

To further investigate the role of central noradrenergic systems in defensive withdrawal behavior, the effects of a2-stimulation were assessed using the a2 agonist isoproterenol. Isoproterenol (1-10 µg iv) produced a dose-dependent increase in defensive withdrawal, significant at the 10 µg dose. L-Propranolol, a non-selective a2-adrenoceptor-induced defensive withdrawal, suggesting that the response to isoproterenol resulted from the activation of a2-adrenergic receptors. These results support earlier data indicating the importance of a2-adrenergic receptors in stress-related behavioral responses (Yang et al., 1990).

Supported by a grant from NINDS (NS 27283)

441.16

INTERACTION OF CEPHRINE AND HOMOCYSTEIC ACID ON ULTRASONIC VOCALIZATIONS AND OTHER FEAR-RELATED BEHAVIORS IN ADULT RATS. D.J. Keene, D.M. Kennedy, V. Ahmad, J. Stern, & L.A. Pohorecky, Center of Alcohol Studies and Department of Psychology, Rutgers University, New Brunswick, NJ 08854-0969.

Previous experiments in this laboratory have found that the administration of 1-3 air puffs to rats elicited robust 22 kHz ultrasonic vocalizations (USVs) which continued with further stimulation, even up to 20 minutes. In the present experiments, the ability of pharmacological agents to modify ultrasonic vocalizations (USVs) and other fear or anxiety-related behaviors was studied in male Long-Evans rats. Previous investigators reported that the 5-HT1A agonist, 8-OH-DPAT, could produce fear-like behaviors and other emotional responses. Our investigations, the peripheral administration of the non-selective 5-HT1A agonist, 8-OH-DPAT, to the dorsal periaqueductal gray (DPAG). In our investigations, the peripheral administration of the 5-HT1A agonist, 8-OH-DPAT, to the dorsal periaqueductal gray (DPAG) produced a behavioral response characterized primarily by immobility and USVs which were geprine reversible (38 nmol/25 µL), while more doral administration induced contralateral rotations or no response. Higher doses (2-5 nmol) or administration more ventral or ventralralateral resulted in violent defensive reactions or running, which were generally incompatible with USVs and geprine (30 nmol/25 µL). Furthermore, administration of these doses elicited running fits which were apparently irreversible (30 nmol/25 µL). These data suggest that 8-OH-DPAT may have different effects depending on the level of the DPAG.
642.1 THE EFFECT OF CONTINUOUS AND INTERMITTENT LEVODOPA ADMINISTRATION ON STRIATAL DOPAMINE METABOLISM: A MICRODIALYSIS STUDY. J.L. Juncos*, M.S. Hooks, J.B. Justice, Jr.. Departments of Neurology and Chemistry, Emory University, Atlanta, GA 30322.

The effects of continuous levodopa administration are currently being studied. However, the effects of intermittent levodopa administration are less well defined. In this study, the effects of continuous levodopa administration on striatal dopamine metabolism was investigated.


Female rats on chronic neuroleptics show a transient increase in postsynaptic D2 DAR density (TRANS rats). In contrast, overrotonized (OXV) rats show hyperlocomotion, the DAR supersensitivity is permanent (PERM rats). We hypothesized that an upregulation of postsynaptic autor occurs in PERM but not TRANS rats. This autor SS should lead to lower DA levels, and thereby decrease motor activity. We confirmed that autor SS is permanent and that it decreases DA levels in PERM rats.


Chronic treatment of rats with haloperidol (HAL,0.25-1mg/kg, S.Wks) increased the number of D-2 receptors in the striatal slices, while no increase was observed by that with risperidone (1mg/kg), an atypical antipsychotic drug that has high affinity at serotonin (5-HT) 2C receptors. HAL-induced elevations in dopamine levels were 59% higher in the continuously-treated group compared to the other two groups (p<0.001) which did not differ from each other. This difference in the levels of dopamine was evident in the 60-180 minute interval post challenge. 5-Hydroxy-tryptophan (5-HTP, 200 mg/kg) and 6-hydroxydopamine and then injected with a single dose of N-ethoxycarbonyl-2-ethylthio-dihydroxyline (EEDQ) which irreversibly inactivates both D1 and D2 receptor sites. D1 and D2 receptors were analyzed at various times after EEDQ treatment. D1 and D2 receptor mRNAs were analyzed by high resolution autoradiography using radioiodinated 5-HT receptor sites, both of which had no significant effect on the number of D2 receptor sites. The results suggest that chronic periodic levodopa administration have a differential and probably reciprocal effect on extracellular dopamine metabolism by the dopamine autoreceptor and the dopamine transporter.

642.4 DERENATION OF MOUSE STRIATUM DECREASES D3 RECEPTOR mRNA AND THE RATE OF SYNTHESIS OF D1 AND D2 RECEPTORS. ZH. Qin, JF. Chen and B. Weist. Div. of Neuropsychopharmacology, Dept. of Pharmacology, Medical College of PA, Philadelphia, PA 19129.

To study the effects of dopaminergic input to the corpus striatum on the expression of D1 and D3 dopamine receptors, mice were bilaterally lesioned with 6-hydroxydopamine and then injected with a single dose of N-ethoxycarbonyl-2-ethylthio-12-hydroxyline (EEDQ) which irreversibly inactivates both D1 and D2 receptor sites. D1 and D2 receptors and D1 and D2 receptor mRNAs were analyzed at various times after EEDQ treatment. D1 and D2 receptors were analyzed in situ by receptor autoradiography, using [3H]-SCH23390 and [3H]-radioligand for D1 and D2 receptors, respectively. D1 and D2 receptor mRNAs were analyzed by high resolution autoradiography using radioiodinated oligodeoxynucleotide probes. The results showed that 4 hr after EEDQ treatment, more than 90% of D1 and D2 receptors were inactivated. The rate of recovery of D1 receptors following inactivation by EEDQ was lower in the lesioned striatum than in the unlesioned striatum while the rate of recovery of D2 receptors was higher in the lesioned striatum than in unlesioned striatum. Denervated striata showed small but significant decreases in D1 receptor mRNA and increases in D2 receptor mRNA. In both cases, the largest percentage changes were seen in the lesioned striatum than in the unlesioned striatum.

Dopamine (DA) D1 receptor density was previously shown to increase after chronic estradiol treatment and to fluctuate during the estrus cycle. In order to investigate the mechanism of the hormonal modulation of D1 DA receptors, the present study investigated brain D1 receptor mRNA changes during the estrus cycle. The level of DA D1 mRNA in the rat striatum and nucleus accumbens was evaluated by in situ hybridization during the estrus cycle and compared to ovariectomized (OVX) rats. During the estrus cycle, rats in the morning of estrus, diestrus I, diestrus II, and proestrus were studied as well as rats in the afternoon of proestrus. A group of rats was OVX and killed 14 days after their surgery. A fragment from the rat striatal D1 receptor cDNA corresponding to the carboxy terminus tail of the receptor was subcloned of the 3' untranslated area was subcloned into pBR322. 18S labelled antisense- or sense-strand RNA probes were prepared by in vitro transcription and hybridized with 35S-labelled 'dextran fixed adult coronal rat brain sections (10 μm). In OVX rats, a high level of DA D1 receptor mRNA was observed in striatum, nucleus accumbens and olfactory tubercle whereas no signal was detectable in the substantia nigra. During the estrus cycle, the levels of D1 mRNA in the striatum and in the nucleus accumbens were decreased in OVX rats as compared to rats in the other stages of the cycle and to OVX rats. The levels of D1 mRNA being similar in the latter, fluctuation of striatal D1 DA receptors showed a peak in diestrus I and of striatal D1 mRNA during the estrus cycle probably reflected a different mechanism of action of steroid hormones on these dopaminergic components. Supported by a MRC of Canada grant to T.D.P.


Changes in dopamine (DA) receptors in basal ganglia result from many movement disorders. Injection of 6-hydroxydopamine (6-OHDA) in the caudate-putamen (CPU) of the rat has been proposed as an animal model for Huntington's disease. We examined the effect of unilateral IA lesions on DA receptor binding and message on both sides of the corpus striatum of male LE rats and the second group with IA (20 μg/2 μl) in the CPU. After 2 weeks, in situ hybridization and RI receptor binding were performed using [35S]ATP labeled DA D1 receptor oligonucleotide probe and [3H]raclopride. A significant increase in D1 receptor binding, (+13%) with no change in receptor binding, was found on the CPU on the contralateral side when compared to saline injected animals. These results suggest that compensatory changes may be occurring on the uninjured side of the brain which involves regulatory subunits of cAMP dependent kinase. This observation of bilateral interdependence in the DA system may be of importance in understanding movement disorders.

642.8 DOPAMINERGIC CHARACTERISTICS IN NEUROBLASTOMA CELL LINES FOLLOWING DIFFERENTIATION WITH RETINOIC ACID. M. Allard, M. Labbé and P. Faillade. CHUL, and School of Pharmacy, Laval Univ., Québec, Canada, G1V 4G2.

Neuroblastoma cell lines, SK-N-MC and LA-N-1, have been found to contain a D-2 dopamine subtype receptor. The neuroblastoma cell lines, SK-N-AS and SK-N-SH, have been found to contain a functional D-1 dopamine subtype receptor that tightly couples to adenylate cyclase. Attempts were made to determine the relationship between these alterations and behavior (amphetamine-induced rotations). It was observed that D2 receptor binding in the ipsilateral (grafted) side was mostly correlated with the degree of behavioral recovery observed. Recovery was also found to be significantly related to the alterations of dopamine uptake sites on the ipsilateral side, but not on the contralateral side. There were no significant correlations between behavior and D1 receptors or D3 message. This study suggests that alterations in D2 receptor binding and dopamine uptake sites are related to the behavioral changes in the grafted brain both after behavioral recovery.

642.9 INDUCTION OF EXPRESSION OF ENDOGENOUS D2 Dopamine Receptor in GH4C1 Cell. S. Allard, M. Labbé and P. Faillade. Mol. Endocrinol., CHUL, and School of Pharmacy, Laval Univ., Québec, Canada, G1V 4G2.

GH4C1 cells are subclone of the GH3 cell line (a prolactin-secreting cell line) which do not respond to dopamine agonists. In contrast with GH3 cells (Mussela et al., J. Biol. Chem., 266: 23896-23899, 1991), native GH4C1 do not show any DA receptors when assessed by PCR for mRNA levels or by [3H]piperidine binding. After transfection of pCMVNeo, a plasmid which carries neomycin resistance, all selected colonies expressing neomycin resistance gene showed the presence of both dopamine D2 DA receptor mRNA and [3H]piperidine binding. This D2 receptor was amplied and sequenced. No difference was observed in the coding region of this receptor when compared with the cloned rat D2 dopamine receptor. In the transfected cells, level of binding for D2 receptor range between 150-500 fmol/mg of protein. Moreover, selective D2 agonist such as quipazine can inhibit forskolin- as well as VIP-stimulated cAMP formation by up to 80% in a dose related fashion. Cells grown in presence of isoproterenol (10-5M) for 3 weeks, induced a four fold increase in the amount of [3H]piperidine binding. The other DA receptor in these cells are still not well understand. However this result should be used to develop drug regulation of D2 dopamine receptor. (Supported by MRC grant)
642.11 REGULATION OF THE ALPHA-2C ADRENERGIC AND 5-HT_1A SEROTONIN RECEPTORS BY DEXAMETHASONE IN AN OPOSSUM KIDNEY (OK) CELL LINE. H.S. Blatch*, R.C. Pleau, D.R. Carulli, N.A. Hass and D.B. Bylund. Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68191-6200.

Previous pharmacological studies from our laboratory have characterized the alpha-2C adrenergic and 5-HT_1A receptors expressed in an immortalized OK cell line as the alpha-2C subtype and the serotonin receptor expressed as the 5-HT_1A subtype. Both of these receptors are down-regulated by treatment with their respective agonists and forskolin.

To further investigate the regulation of these receptors in OK cells, we treated with dexamethasone. Cells were grown in serum free, steroid free media (Ultroser SF, Sepracor, Columbia, MD) in the presence and absence of 100 nM dexamethasone. After 72 hours, control and treated OK cells were harvested and membranes were prepared for saturation binding assays. The B_max values were determined for the alpha-2C adrenergic receptor using [3H]rauwolscine and for the 5-HT_1A serotonin receptor using [3H]lodoxamol. The alpha-2C subtype receptor number was decreased by -30% and the 5-HT_1A subtype receptor number was increased by -55% with dexamethasone treatment. Thus, it appears that in OK cells dexamethasone causes a down-regulation of alpha-2C receptors and an up-regulation of 5-HT_1A receptors. (Supported by NISS grants GM07874 and MH47354).

642.12 REGULATION OF A2 ADENOSINE RECEPTOR mRNA BY PHORBOL ESTERS IN THE PC12 CELL LINE. J.S. Fink* and R A Peterfreund. Deps. of Anesthesia and Neurology, Massachusetts General Hospital, Boston, MA 02114.

A rat DNA which encodes a high affinity A2 adenosine receptor (A2R) was recently cloned and expressed in our laboratory (Mol Br Res, 1992, in press). PC12 cells, a clonal rat cell line, were used to express functional high affinity A2Rs (J. Neurochem, 37, 1431: 1981) were found to produce high levels of mRNA which specifically hybridized to a rat A2R cDNA probe. Activation of the protein kinase C (PKC) second messenger system is known to regulate mRNA levels for other G protein-linked receptors. We asked if activiation of the PKC second messenger system regulates A2R mRNA levels in PC12 cells.

Treatment with tetradecanoyl phorbol acetate (TPA), which activates PKC, reduced the levels of A2R specific mRNA by 50-75% in two different subclones of PC12 cells. The effect was dose dependent; inhibition was observed for doses greater than 10 nM. A reduction in A2R mRNA levels was first detected after 2 hours and was maximal by 5-6 hours. Structural analogs of TPA known to have reduced efficacy to activate PKC were less effective in reducing A2R mRNA levels. Cells treated with A2A and allolactone recover overnight exhibited an increase in A2R mRNA levels. The A2R is believed to exert its intracellular effects by a signal transduction pathway involving activation of the production of cAMP, but not through activation of PKC. Our results are consistent with heterologous regulation of transcription or stability of A2R mRNA by PKC activation.


We have previously shown that the βγ subunit of G proteins stimulates the agonist- or light-dependent phosphorylation of muscarinic receptors (mACHRs) and rhodopsin by a protein kinase (mACHR kinase) partially purified from porcine cerebrum (J.Biochem., 267,2222 (1992)). The light dependent phosphorylation of rhodopsin is blocked by pertussis toxin and is restored by the addition of βγ subunit. We hypothesize that the βγ subunit activates agonist dependent phosphorylation of G proteins and thereby facilitates the desensitization of phosphorylated receptors. The CDNA for the mACHR kinase was kindly provided by Dr. R.J. Lefkowitz.


Carbachol (CCh) mediated a stimulation of [3H][IP] accumulation in transfected B82 cells expressing the M1 receptor with an EC50 value of 22.6 nM and a maximal stimulation of 22 fold above basal level. Pretreatment of these cells for 60 min at 37°C with phorbol 12-myristate, 13-acetate (PMA) resulted in a rapid desensitization of the M1 receptor with an IC50 value of 9.1 nM and a maximal inhibition of 84% of the effect of 100 μM CCh. PMA (1 μM) pretreatment had no effect on the multiple affinities of CCh for the M1 receptor in intact cells or membrane preparations (Kd = 2.85 μM; Kd = 389 μM), contrasted with a single low affinity of 124 μM for CCh in the presence of 100 μM GTPγS. Treated cells showed a reduction in total M1 receptor density (67.4 ± 6.6 % of [3H][QNB] binding in control cells) as well as the number of receptors on the cell surface (76.7 ± 3.3 % of [3H][QNB] binding in control cells). The affinity of the antagonist was not affected (p<0.05). The M1 receptors expressed in B82 cells were coupled to an inhibition of cAMP formation and a small stimulation of [3H][IP] accumulation. Neither of these functions nor the density of the M1 receptors was altered by 0.1 μM PMA pretreatment. These data demonstrate clearly a differential sensitivity of the M1 and M2 receptors to PMA in their functional coupling. The rapid desensitization of the M1 receptors may be due to a PMA-induced uncoupling of phospholipase C from the G-proteins. Supported by AHA and NIMH.


Asthma is characterized by the hyperresponsiveness to constrictors and hyperresponsiveness to relaxants. Since most, if not all, receptors for constrictors and relaxants are members of "G protein coupled receptor" family, we hypothesized that an altered expression of G proteins is responsible for such receptor dysfunctions. MIID (acute) and severe (chronic) asthma were produced in previously sensitized guinea pigs by a single or multiple challenges with aerosolized immunogen, ovalbumin. Levels of Gαq, Gα12 and Gα13 but not of Gαw and Gα5 increased as a function of time when measured at 0, 12 and 24 hr postinnominal challenge. Guinea pigs with severe asthma had larger increases in Gαq and Gα12 than animals with mild asthma. The in vitro assay of isolated tracheas showed that the hyperreactivity to cholinergic agonist takes place only in the animals with severe asthma immediately after the challenge, while the hyperreactivity to isoproterenol progressed in both of the animals with mild and severe asthma. Our results suggest that an altered expression of Gq and G1 proteins in airway smooth muscle is partly, if not totally, responsible for receptor dysfunctions observed in asthma.


Alterations in serotonin (5-HT) receptor binding were assessed in male rats by quantitative autoradiography following intrahypothalamic injection of the serotonin 5-HT_1A and 5-HT_1B receptor antagonists WAY 100635 (5,7-DHT) and 5,7-DHT. [3H][OH]-DPAT binding was used to label 5-HT1A receptors and [125I]-iodoazidopindolol binding in the presence of isoproterenol (to mask 5-HT_1B) and [125I]-iodoazidopindolol binding in the presence of isoproterenol (to mask 5-HT_1B) receptors. Seven days after 5,7-DHT, when 5-HT levels are lowest, [3H][OH]-DPAT binding was increased in the ventromedial and dorsomedial hypothalamic nuclei (VMH, DMH) in the same animals, [125I]-iodoazidopindolol binding was increased in the VMH, but unchanged in the DMH. No changes in 5-HT1A or 5-HT1B binding were observed in the lateral hypothalamic area, the dentate gyrus, or the CA1 region of the hippocampus, in spite of a large decrease in the binding of [3H]paroxetine, which labels the presynaptic 5-HT transporter site. These results demonstrate differential regulation of 5-HT receptors in response to denervation and also suggest that some of the 5-HT1B receptors in the VMH are located post-synaptically. Studies in females are in progress to determine possible sex differences in the response of 5-HT receptors to 5,7-DHT. Supported by NS07080.
ARTSENSE OLIGODEOXYNUCLEOTIDE INHIBITION OF NEUROPEPTIDE Y (NPY) Y1-RECEPTOR EXPRESSION, F. Yee*, M. Heilig and C. Wahlestedt. Dept. Neurobiol., Div. Neurobiol., Scripps Res. Inst., La Jolla, CA 92037. The Y1-receptor mediates behavioral and vascular actions of NPY. Since specific Y1-receptor antagonists are not available, we have employed the antisense oligodeoxynucleotide approach to suppress endogenous Y1-receptor protein synthesis in vitro and in vivo (Heilig et al, this meeting). Based on the recent cloning of human and rat NPY Y1-receptor (Larhammar et al., J. Biol. Chem., in press), antisense and/or control oligodeoxynucleotides (D-oligos) corresponding to different regions of the receptor were synthesized. These D-oligos were then added to media of cultured cells, e.g. rat primary cortical neurons and human neuroblastoma cells (SK-N-MC), which had previously found to express the Y1-receptor by RT-PCR and/or Northern analyses (ibid). In both these cell types, antisense D-oligos directed to regions immediately downstream of the initiation codon were found to reduce, by up to 90%, high affinity binding sites labeled by 125I-IVY (PPY). D-oligo concentrations as low as 0.1 μM (maintained over 3-5 days) were sufficient for reducing 125I-PPY binding sites in cortical neurons under serum-free conditions. In contrast, SK-N-MC cells, which were transcriptionally prior to addition of D-oligos and grown in the presence of heat inactivated sera, required 10 μM concentrations for similar suppression. Comparable data (70% inhibition of full NPY response) were obtained when assessing the ability of NPY to reduce forskolin stimulated cAMP accumulation in SK-N-MC cells treated with the antisense D-oligo. No biochemical (protein content and/or localization) abnormalities were observed to be induced by the D-oligos at the above concentrations. The described antisense approach may thus be useful in attempts to specifically affect Y1-receptor synthesis and function, resulting in the reduction of NPY efficacy.

DEVELOPMENTAL EXPRESSION OF CANNABINOID RECEPTOR mRNA C.R. McLaughlin*, W.L. Dewey and M.E. Abood. Dept. of Pharm., Med. Coll. of Virginia, Virginia Commonwealth University Richmond, VA 23298. The cloning of a putative cannabinoid receptor affords the opportunity to examine its developmental expression and distribution. Other receptor systems, notably those for the opioids, have been shown to have distinct developmental time frames. For the initial study, Sprague-Dawley rats from the following age groups were employed: postnatal days 2, 5 and adults. The brains were grossly dissected into cerebellum and forebrain, and total RNA was extracted by a modified acid-extraction method. Measurements of cannabinoid receptor mRNA was analyzed by two methods: Northern blot analysis and polymerase chain reaction (PCR). The probe used in the Northern blot analysis was a full length cDNA corresponding to the rat cannabinoid receptor. The probe was cloned in our lab based on published sequence information. Oligonucleotides primers based on bp 1-21 and bp 824-843 on the opposite strand were chosen for use in the PCR. Preliminary results indicate that postnatal day 2, the cannabinoid receptor mRNA can be detected in the brain. In addition, splenic cords from 13 day old rats were analyzed. Cannabinoid receptor message was present, but at low levels, consistent with published receptor autoradiographic data from adult animals.

This research was supported by DA05274 and DA07027.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: STRESS

463.1 FORCED AMBULATION IN AN EXTREME POSTURE INCREASES TONIC CORTICOSTEROID BUT NOT ACTH LEVELS IN RATS. J. Bhatnagar, J. Bhatnagar, R. Bhatnagar. VA Medical Center (131), San Diego, CA 92161. An animal model of low back dysfunction has been described (1) (ibid). Now serum levels of corticosterone (B) and ACTH after six weeks of forced ambulation are reported so as to assess the degree of stress between groups of rats. Three groups of rats were compared: rats forced to ambulate on a flat surface (normal posture), rats forced to ambulate in rotating cylinders (extreme posture), and rats not forced to ambulate. After six weeks, rats forced to ambulate in cylinders and rats not forced to ambulate had higher tonic levels of B than rats forced to ambulate on a flat surface. The relationships of tonic levels of ACTH to B were different between the rats forced to ambulate in cylinders and on a flat surface. It is suggested from the results that forced ambulation in a normal posture lowers tonic levels of B, and forced ambulation in an extreme posture is more stressful than in a normal posture. Since the adaptation to exercise stress was associated with higher B levels when exercise was performed in an extreme posture, the voluntary adoption of an extreme posture in the low back is stressful in exercising (forced ambulation) rats.

463.2 GLUCOCORTICOID REGULATION OF THE ADRENOMEDULLARY CATECHOLAMINERGIC SYSTEM FOLLOWING A MILD ACTH INJECTION. L. Bhanagar, J.B. Mitchell, J. Diorio & M.J. Meaney. McGill University, Deps. of Psychiatry, Pharmacology, and Neurology & Neurosurgery, Douglas Hospital Research Center, Montreal, Quebec H3H 1Y9, Canada. Adrenal modulatory enzymes are regulated both by spainchon innervation and by glucocorticoids (GCs). Phenylethanolamine N-methyltransfase (PNMT), the final enzyme in the catecholaminergic bioplastic pathway, is predominately regulated by GCs. Changes in the activity of PNMT in rats have been observed following a variety of chronic stressors, as well as with more acute stresses, such as an intermittent 2h swim stress or a 2.5 h immobilization stress. We have previously shown that bovine adrenomedullary cells respond to a pulse of GCs as early as 15 min by increasing PNMT 2-3 fold (Betito et al, 1992, J. Neurochem 58, 1853). In the present study, we looked at the adrenomedullary response of male rats to a more moderate acute stress, 20 min restraint stress, where the increase in plasma corticosterone (CORT) is relatively short (1h). A significant increase in PNMT activity was observed only at 18h (17%) and 24h (14%) following restraint, but not before. Adrenalectomy and nonsteroidal agents of the adrenal increased significantly at 18h with no increase in tyrosine hydroxylase (TH). Splanchic nerve transection did not alter basal PNMT and TH activities (as previously shown) and did not prevent an increase in PNMT activity following restraint. Inhibition of CORT synthesis by metyrapone (100 mg/kg, injections 24h and 2h prior to stress) prevented a stress-induced increase in PNMT activity. Adrenalectomy and nonsteroidal agents of the adrenal increased significantly at 18h with no increase in CORT activity. In this study, the increase in PNMT activity was associated with increased TH activity and PNMT activity was inhibited by metyrapone. The results demonstrate that a relatively short moderate stressor, which elevates GCs for only 1h, can elevate the activity of PNMT following a period of 18-24h. The regulation of this activity appears to be independent of neural input and dependent on the release of CORT during the period of stress.

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We have previously shown that the HPA axis in the female rat is most sensitive to stress during the proestrus phase of the estrous cycle (Vainio and Meaney, Endocrinology, 1990). This suggests that physiological levels of stress hormones during proestrus could regulate ACTH co-secretagogues. We examined median eminence CRH and ACTH content during the estrous cycle and in overconditioned rats with high and low-reared females. CRH and ACTH content was higher in proestrus-obese and lower in proestrus-athletic rats compared to diestrous: CRH=11±2 vs. 7.2±1.3, and 5.3±0.4 pg/mg protein, respectively. These differences were not statistically significant (p>0.05). However, we observed a trend for the proestrus-obese group to have lower CRH and higher ACTH content compared to the other two groups. These results suggest that the proestrus phase of the estrous cycle may be a critical period for the regulation of the HPA axis and its sensitivity to stress.

643.4 EFFECTS OF ACUTE RESTRAINT STRESS ON CORTICOSTERONE, ACTH, CRH, AND VASOPRESSIN DIFFER ACROSS SEX AND STRAIN. S.J. Cummins, A.C. Griffith, and C.C. Whisnant. University of California Davis, CA 95616 and The Ohio State University, Columbus, OH 43210.

Experimental autoimmune encephalomyelitis (EAE), a model for the human demyelinating disease multiple sclerosis, is frequently studied in the highly susceptible Lewis (LEW) strain of rat. We and others have reported that stress delays the onset and decreases the severity of EAE, particularly in the LEW female. The hypothalamic-pituitary-adrenal axis (HPA) has been hypothesized to play a role in the innate as well as stress-induced resistance to various autoimmune diseases, including rheumatoid arthritis and EAE. Therefore, we have examined corticosterone (CORT), adrenocorticotropic hormone (ACTH), corticosteroids releasing hormone (CRH) and vasopressin (VP) levels in response to stressors in both sexes of 4 rat strains with varying susceptibility to inflammatory disease (LEW, Lewis Resistant, Brown Norway, and Fischer (F344)). Plasma CORT and ACTH levels were determined by radioimmunoassay, and expression of CRH and VP mRNA in the hypothalamic-pituitary-nervous system (HPNS) was examined using in situ hybridization histochemistry. CRH and VP levels in the median eminence (ME) were visualized immunohistochemically. Levels of CRH and VP mRNA within the brain were similar across strains in non-stressed animals, though stress increased VP mRNA levels in the ME of the highly resistant F344's consistently more than in the susceptible LEW. In response to 30 min of restraint stress, CORT and ACTH levels increased in both sexes of all strains, but to a greater degree in F344's than LEW's. CORT increased to higher levels in females than males, regardless of strain. After 30 min of stress, small increases in CRH and VP mRNA levels were observed in the PVN of F344's, but not LEW animals. Taken together, these stress data suggest that stress-induced changes in CRH and VP expression are related to the differential genomic regulation of the HPA axis by stress.

643.5 PITUITARY-ADRENOCORTICAL AND ADRENOMEDULLARY RESPONSES TO A NOVEL STRESS IN CHRONICALLY COLD STRESSED RATS. K. Becker, S. Shugerman, J.B. Mitchell, P. Bayes, and M.J. Meaney. McGill University, Montreal, Canada H4H 1R3.

Chronically stressed rats exhibit exaggerated responses to hypothalamic-pituitary-adrenocortical and adrenergic systems in response to acute stress. Basal levels of hypothalamic enzymes (phenylethanolamine-N-methyltransferase, PNMT; tyrosine hydroxylase, TH), catecholamines (epinephrine, E; norepinephrine, NE), and pituitary-adrenocortical hormones may or may not be elevated depending on the chronic stress. Therefore, adrenal medullary and adrenal cortical systems may not respond to acute stressors ether than ADX appears to be attenuated in both mothers and pups. Supported by Can. MRC (M.W. and D.M.N.)


Current models of stress-induced hypothalamic-pituitary-adrenocortical and adrenergic dysregulation hypothesize that the hypophyseal-pituitary-adrenocortical system (HPA) may play a role in the regulation of stress-induced resistance to various autoimmune diseases, including rheumatoid arthritis and EAE. Therefore, we have examined the effects of a high fat diet on the expression of CRH and VP mRNA in the hypothalamic-pituitary-nervous system (HPNS) in young male rats. We have previously shown that the HPA axis in the female rat is most sensitive to stress during the proestrus phase of the estrous cycle (Vainio and Meaney, Endocrinology, 1990). Taken together, these results suggest that estrogen enhances the HPA axis response to stress during proestrus by enhancing the synthesis/secretion of CRH and, possibly to a greater extent AVP as an o1 adrenergic agonist.

643.7 MODEST INCREASE IN NADIR CORTICOSTERONE (B) SECRETION IN OBSESE (fa/fa) Zucker Rats ALTERS FEEDBACK INHIBITION ON ACTH RELEASE. C.D. Walker, J.S. Stern, L.S. Myers*, M.F. Dallman. Dept of Physiology, UCSF; Dept of Nutrition UCSD, Dept of Psychology, CSU Stanislaus, CA 95362.

Genetically obese Zucker (fa/fa) rats exhibit a number of metabolic and endocrine disorders, most of which can be reversed by adrenalecromy (McClung et al., Endocr., 110:1676). We studied basal adrenergic and hypothalamic activity in intact lean (Fa/Fa) and obese (fa/fa) rats and the ability of B to affect ACTH and insulin secretion and fat deposition in both sexes. The effects of ACTH, glucagon, and B on food intake, body weight, and food intake were measured on postnatal day 18. At postnatal day 18, ACTH secretion and feeding behaviors were observed in both sexes. Hyperinsulinemia in obese rats did not show greater increases in insulin secretion with increasing B than lean rats. Fat deposition slightly increased with B in obese, but not in lean rats. Like many models of chronic stress, small increases in natal B secretion in obese rats are associated with a decline in feedback sensitivity on basal ACTH secretion and subtle changes in the central regulation of ACTH secretion occur to maintain homeostasis. Supported by Swiss NRF (CDW), DK28172 (MFD), DK18899 (JSS).
PATTERN OF IMMEDIATE EARLY GENE ACTIVATION IN RAT BRAIN FOLLOWING ACUTE STRESS. 1 W.E. Collinson*, 2 L.P. Horwich, and 3 S.L. Watson. 1University of Michigan, Mental Health Research Institute, Ann Arbor, MI, 48109-0720, and 2University of Kentucky Medical Center, Dept. Anatomy and Neurobiology, Lexington, KY, 40536-0694.

In an effort to produce a functional map of the neural circuitry related to activation of the hypothalamic-pituitary-adrenal axis, we examined the pattern of induction of the immediate early c-fos gene in rat brain at 30, 60 and 120 min. following acute immobilization stress. A radiolabelled cRNA probe was used to detect c-fos mRNA using in situ hybridization histochemistry. Results indicated regionally specific patterns of c-fos expression. Prior to stress c-fos was undetectable in most brain areas, and was markedly induced at 30 min. post-stress in the cingulate, infralimbic and orbitofrontal cortices, the piriform cortex, and in several neocortical areas including frontal and parietal regions. Also prominently labelled at 30 min. were the lateral septal nucleus, portions of the lateral hypothalamus, the hypothalamic paraventricular nucleus, the mammillary hypothalamic region, the medial and cortical amygdaloid nuclei, as well as a number of thalamic and brainstem regions. In the majority of these regions message levels were reduced at 60 min., and generally undetectable at 120 min. Exceptions included the piriform and parietal cortices, which exhibited a slower rate of decline, remaining detectable at 120 min post-stress. We are currently examining the induction of c-fos in these regions at earlier and intermediate time points, and have begun characterizing other immediate early genes (cjun, elk/a268) with respect to chemically defined neuronal populations. Supported by DA02265, MH422251, and ST-32DK07245.

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ALTERED CORTISOL RESPONSE TO STRESS AFTER FOUR MONTHS' PRACTICE OF THE TRANSCENDENTAL MEDITATION PRACTICE. 1 E.K. McLean, 2 K.O. Wallace, 3 J.M. Benson, 4 J.E. Mandarino, 5 R. Watt4 and 6 R.H. Schneider. Dept. of Physiology and Pharmacology, Maharishi International University, Fairfield, IA 52556 and 7University of Iowa, Iowa City, IA 52242.

Recent studies on the wild baboon suggest that low basal cortisol levels and high corticosteroid response to stressors is a more reliable, more adaptive profile than the opposite. Previous research on the Transcendental Meditation (TM) technique has reported decreased basal cortisol levels both acutely and longitudinally. Using a random-assignment, pre-post design, the present research examined before and after the effects of four month's of TM or stress education class (SEC) on basal cortisol levels and the dynamic response of plasma cortisol to laboratory stressors. Twenty-nine healthy Caucasian males (ages 18-32) were randomly assigned for 4 months to either TM or SEC. Each subject was sampled using a continuous withdraw pump (Dakamed) during a one-hour laboratory stress session (7-10 min) which included a minute task (bpm) that was administered by computer (A&M). Plasma was assayed for cortisol by RIA (DPC) and statistically analyzed by t-test and ANCOVA. The decrease in basal cortisol from pretex to posttest was significant for the TM group when compared to the SEC group (t(21.5) = 2.21, p = 0.043). In addition, the TM group exhibited a significant increase in ACTH response to stressors. These changes are consistent with a decrease in hypercortisolemia in both basal and post-stress conditions. These results suggest that TM may be a prophylactic site for regulation of the pituitary-adrenal axis. (Supported by NIH RR0167.)
643.15

ADRENOCORTICAL FUNCTION IN PTSD
Michele Murburg M.D.* University of Washington, VAMC Seattle

Patients with Post-traumatic Stress Disorder (PTSD) have been found to have lower 24-hour urinary free cortisol levels than do controls. To test whether PTSD patients have less cortisol available for release in response to maximal stimulation by ACTH, we administered the ACTH analog Cosyntropin to healthy, medication-free male patients with PTSD and controls. On the study day, i.v. catheters were inserted and 45 minutes later 3 basal blood samples for cortisol were drawn at 10 minutes each. 0.25 mg, was given i.v., and then cortisol levels were drawn at 15, 30, 60, 90, 120, 150, 180 and 210 minutes. T-tests revealed no differences in RIA-determined basal plasma cortisol (7.8+1.9 vs. 10.5+2.7 mg/dl), or area under the cortisol curve 437+366 vs. 3265+1197 mgxmin/dl) for PTSD (N=6) or control subjects (N=4) (mean±SD). With the small N tested, there is no evidence for a smaller cortisol response to maximal stimulation with ACTH in PTSD.

644.1


Although a wealth of knowledge exists regarding the morphologic development of the barrel cortex and thalamocortical relations, very little is known about the development of functional activity in these regions. In order to further understand mechanisms that participate in development of the barrel field, mice (ages 1 wk to 6 mos) that were either normal or unilaterally basal-forebrain-lesioned (BFL) at birth underwent a 2-deoxyglucose (2DG) experiment. Following the 2DG injection, each mouse was either returned to its cage with whiskers intact, or received bilateral stimulation to a single whisker. At one wk of age, for both normal and BFL mice, although barrels were fully formed as indicated by cytochrome oxidase staining, above background stimulation-evoked 2DG activity was difficult to visualize in the barrel field. At this age, the label was distinctly different from the pattern evoked in the adult and occurred in layer 4, the supragranular layers, and layer 6, with a distinct activity gap in layer 5. Activity specifically elicited by stimulation of single vibrissae was in smaller areas than comparable activity evoked in the adult. At 2 wks of age, the whisker-stimulated activity was greater than that observed in the 1 wk animals, but was not close to the adult pattern until 4 wks of age. In contrast to weak activity observed in the barrel field, the ventrobasal thalamus demonstrated a remarkably high level of 2DG uptake, which was significantly stronger in the 1-2 wk old animals compared to the 4 wk old animals. At early developmental ages 2DG uptake in the somatosensory cortex ipsilateral to the BFL did not significantly differ from the contralateral or normal hemispheres. Supported by RO7064.

644.2

DEVELOPMENT OF NON-NMDA GLUTAMATE RECEPTORS IN RAT BARREL FIELD CORTEX. M.E. Blue1, M. Fossati2, T.M. Dawson1, S.H. Snyder3, and M.V. Johnston. Kennedy Krieger Research Institute and Johns Hopkins School of Medicine, Baltimore, MD, and Neuroscience and The Johns Hopkins Univ. Sch. of Med. Baltimore, MD 21205.

The ontogeny of non-NMDA (AMPA) and metabotropic (mGluR) glutamate receptors in barrel field cortex was studied in cats between postnatal ages 14 and 57 and in adults. To examine mGluR sites, flattened sections of cortex were labeled with [3H]glutamate and NMDA displacers. Autoradiographically labeled mGluR sites form a vibrissa-related map at P4, with higher densities of receptors in barrel centers than in barrel septa or surrounding tissue. Densities of mGluR sites in barrel centers were highest at P10 and then declined with age. At P17 the density in barrel septa and surrounding cortex was 62% (2.8±0.5 pmol/mg protein), in the adult, it is only 4±1% (1.9±0.5 pmol/mg protein). The ontogeny of mGluR sites was also examined using an affinity purified antibody to the mGluR receptor which recognizes both a and b isoforms. Staining of mGluR receptors is enhanced in the immature brain, with greater concentrations of mGluR1 immunoreactivity in barrel centers than in septa and surrounding cortex. Within barrel centers, numerous varicose processes (presumably axonal) are stained. Relatively few neurons within the barrels are stained compared to surrounding cortex; these immunoreactive cells are predominantly located in barrel walls and are often contacted by immunoreactive boutons. In the adult, the relatively low density of staining in barrel contrasts with that in surrounding cortex; many fewer neurons and processes are stained. Immunoreactive, labeled with [3H]-AMPA, show a contrasting developmental pattern of expression to that of mGluR sites. At all ages examined, barrel centers contain fewer AMPA-like receptors than barrel septa or surrounding cortex. The density of AMPA sites increases to its highest value at P17 (1.9±0.5 pmol/mg protein) and then declines slightly to the adult value (1.7±0.5 pmol/mg protein). These results show a differential ontogeny of AMPA and mGluR receptors. Based on distribution and timecourse, the mGluR sites appear more likely to influence the process of barrel formation. Supported by NIH grants NS28208 and NS29167.
Changes in corpus callosum inputs to barrel field during postnatal development in albino rats revealed by DI. L. L. Mangini, J. A. Kibbee, B. E. Cooper*, Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis, TN 38163. Dept. of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY 40292.

In adult albino and pigmented rat SI cortex, the corpus callosum inputs to the barrel field in layer IV were shown to fill the septae outlining the barrels (Koralek et al., J. Comp. Neurol. 1991). The developing CC projections to rat SI cortex have not been examined in tangential sections of the somatosensory corthic (TC) inputs to the barrel field of albino and pigmented rats have shown that TC inputs fill the hollow core of the barrel from postnatal day (PND) 2 (of birth/PND 1) to adult (e.g., Ihng and Cooper, Ann. NY Acad. Sci. 1991). In the present study we examined the developmental changes in CC inputs to the barrel field by placing crystals of the cyanine dye, Dil, in the mid-sagittal CC in aldehyde-fixed tissue from PND 1 to adult in albino rats. Tangential sections 150 μm thick were examined with epifluorescence using a standard rhodamine filter set. The CC inputs do not form a detectable barrel pattern until after PND 5. By PND 9 the CC input takes the form of filled hollows throughout the barrel field. At PND 14 and 17 CC inputs were a mix of transition, filled hollows in the anterolateral 2/3 and filled septi in the posteromedial 1/3 of the barrel field. At PND 24, 27 and adult the CC barrel field input is restricted to filled septae. Thus, the barrel field in albino rats changes substantially during development; results from pigmented rats will be compared. Supported by NIMH grants RO1-NS-14666 (JAB) and RO1-NS-27068 (NGFC).

Evidence for rapid changes in the receptive field organization of SI cortex in squirrel monkey: An intracellular recording study combined with reversible deafferentation. R. S. Water*, C. L. Deit. Dept. of Anatomy and Neurobiology, UT, Memphis, Coll. of Medicine, Memphis, TN 38163.

The ability of the cortex to rapidly reorganize following peripheral nerve injury suggests the unmasking of previously undetected inputs. We examined intracellularly recorded evoked responses in SI cortex following reversible deafferentation by ablating the nerve and examined SI cortex for changes in receptive field organization. We report that removal of peripheral nerve input from a single nerve alters the responsiveness of SI neurons and that the effect is reversible. A adult squirrel monkey was anesthetized with ketamine (50mg/kg), the head and arm were stabilized in custom made holders, and the unanesthetized side was exposed and placed on a pair of cuff stimulating/recording electrodes; the nerve was also snared and placed on a modified cooling device. Following forepaw preparation, the contralateral forepaw was exposed, and an acrylc recording chamber was fashioned on the remaining bone. Carbon fiber electrodes were used to record evoked responses. Evoked responses were recorded by mechanical and/or hand held stimulators, and the receptive field was reexamined. Using identified in terms of suprathreshold and subthreshold components. The mechanical evoked responses were recorded from the forepaw using an implanted electrode in a squirrel monkey anesthetized during recording. Subsequent recordings were conducted during acute penetrations in an awake (conditioned) macaque. In both monkeys, compared to coloconic median nerve responses, radial nerve responses typically had longer latencies (by 11-16 msec), lower amplitude, longer duration, more variability and different laminar distribution. Since median and radial nerve primary cortical responses differ by only 1.8 ms, 1) the latency difference is not due to differences in median and radial nerve conduction times to cortex; 2) the "delayed" radial nerve response is not due to a trivial cause such as volume conduction. Rather, it may reflect indirect or extraneural input. (MH07253 and DC00657).

Somatosensory cortex and thalamocortical relationships: development and plasticity.
THE ORGANIZATION OF SOMATOSENSORY AREA 3A IN THE NEOCORTEX OF THE FLYING FOX (PTEROPUS POLIOCEPHALUS)
S. Firnigan, L. Knobler, J. C. Clancy, and M. Calford. VTHRC, Department of Physiology and Pharmacology, University of Queensland, Australia 4072.
Although there is some evidence for the existence of a deep representation rostral to 3b in a number of mammals, there are no descriptions of the overall organization of this deep rostral representation that include all modalities. Because neurons in 3a respond to joint manipulation and hard taps to the body surface, it is likely that this is a primary representation of motor control. Thus, how this field is organized and interconnects with cutaneous representations and motor cortex is of great interest.

The flying fox was chosen to study the organization of area 3a because it has a well-known architecture. First, as an achetaodon, the flying fox shares a close phylogenetic relationship with primates, and information about somatosensory reorganization in the human cortex in response to peripheral deafferentation and not the absolute amount of deafferented cortex limits the extent of reorganization that is possible. (Supported by NIH NS16446 and HD101052.)

The precise role of the corpus callosum in connecting the two cerebral hemispheres is unknown. However, we do know that the corpus callosum fuses midline representations in somatosensory and visual systems so that areal patterns of connections could be observed. Connections of area 1/2 were relatively dense (see Krubitzer et al., this meeting). To determine the extent of interhemispheric reorganization after nerve injury, we investigated the connections of area 1/2 in one hemisphere only, it is likely that these plastic changes are transferred to the ipsilateral hemisphere via the corpus callosum. However, in the animal under study (flying fox), the callosal connections of areas 3b and 3a are sparse, while those of an adjacent and interconnected somatotopically-organised field (area 1/2) are relatively dense (see Knobler et al., this meeting). To uncover the pathway(s) responsible for the interhemispheric reorganization, focal plasticity, focal cooling experiments were performed in adult, keamine-anaesthetised flying foxes (Pteropus ptilocerus). Cooling the forelimb digit (D1) representation of area 3b, until neural activity beneath the cooling probe decreased, resulted in an expansion (of approximately 2-4 times in area) of a D1 receptive field (RF) recorded from an extracellular microelectrode in area 1/2 in one hemisphere was cooled and rewarmed (n=5). In two other animals, this effect was observed only in the area 3b RF ipsilateral to the cooling probe. These results suggest that blocking the callosal pathway(s) produces a disinhibition that allows unmasking of large RFs and is probably mediated by the interhemispheric connections of area 1/2 via its intercalational connections with area 3b.

When multiple digit amputations are made to the same area of the body, the resulting cortical reorganization is spatially incomplete. However, when a much larger area of cortex is deprived by transection of the median and ulnar nerves, deafferenting the entire voluntary surface of the hand [PNAS, 88 (1991) 6976], apparently complete reorganization ensues, with the dorsal of the hand and digits expanding their representations. It is possible that incomplete reorganization occurs after multiple amputations because both the dominant and preferred latent set of inputs have been removed (i.e., the glabrous and hairy surface of given digits). We report here on squirrel monkeys in which the ulnar and radial nerves were transected and ligated. This manipulation completely eliminates both glabrous and hairy surface inputs from D6, ulnar D4, and the ulnar hand. Yet the total amount of cortex deprived is less than with median and ulnar nerve cut. We find in these animals, as in monkeys with multiple digit amputations, that cortical reorganization in areas 3b and 1/2 is incomplete. Large sectors of deprived cortex are unresponsive to cutaneous stimulation, though noncutaneous stimulation can frequently drive responses. Thus, the specific pattern of peripheral deafferentation and not the absolute amount of deprived cortex limits the extent of reorganization that is possible. (Supported by N.I.H NS16446 and HD101052.)

The precise role of the corpus callosum in connecting the two cerebral hemispheres is unknown. However, we do know that the corpus callosum fuses midline representations in somatosensory and visual systems so that areal patterns of connections could be observed. Connections of area 1/2 were relatively dense (see Krubitzer et al., this meeting). To determine the extent of interhemispheric reorganization after nerve injury, we investigated the connections of area 1/2 in one hemisphere only, it is likely that these plastic changes are transferred to the ipsilateral hemisphere via the corpus callosum. However, in the animal under study (flying fox), the callosal connections of areas 3b and 3a are sparse, while those of an adjacent and interconnected somatotopically-organised field (area 1/2) are relatively dense (see Knobler et al., this meeting). To uncover the pathway(s) responsible for the interhemispheric reorganization, focal plasticity, focal cooling experiments were performed in adult, keamine-anaesthetised flying foxes (Pteropus ptilocerus). Cooling the forelimb digit (D1) representation of area 3b, until neural activity beneath the cooling probe decreased, resulted in an expansion (of approximately 2-4 times in area) of a D1 receptive field (RF) recorded from an extracellular microelectrode in area 1/2 in one hemisphere was cooled and rewarmed (n=5). In two other animals, this effect was observed only in the area 3b RF ipsilateral to the cooling probe. These results suggest that blocking the callosal pathway(s) produces a disinhibition that allows unmasking of large RFs and is probably mediated by the interhemispheric connections of area 1/2 via its intercalational connections with area 3b.

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Does performance of tactile texture discrimination depend on scanning velocity?

Eliad Ahissar* and Merav Ahissar.

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The ability of humans to actually recognize or discriminate between different textures improves when they are allowed to move their fingers across these surfaces. Several research groups have claimed that the velocity of movement is irrelevant to the subject's performance. Yet, this claim was made in studies that did not allow subjects to utilize their own scanning strategies. Previously, we suggested (model of the "PIL model") predicting that when the task becomes difficult, requiring subjects to match optimal performance, scanning velocity (VS) will depend on the spatial frequency (SF) of the texture (Ahissar and Vaadia 1999 Proc. Natl. Acad. Sci. USA 96(22): 8935-8939). The model further predicts that scanning velocity will change in a way that will keep the resulting temporal frequency (f = VS / SF) as close as possible to a preferred value. Such three preferred values were suggested, as expected from the tri-modal distribution (0-15 Hz, 15-50 Hz and 80-250 Hz) of the oscillating frequencies of local cortical oscillators found in the second somatosensory cortex of the monkey.

The distribution corresponds also to the distribution of the best frequencies for activating mechaonocereceptors in the finger tips of humans and monkeys.

Naive human subjects were tested for tactile recognition and discrimination tasks. The subjects were allowed to choose their own scanning strategies. The locations of the scanning fingers of both hands were sampled in 20 ms time resolution and 0.1 mm spatial resolution using an ultrasonic location detector. The analysis of scanning strategies revealed that when the task became difficult subjects adjusted their finger velocities to the spatial frequencies, as predicted by the PIL model.
ACTIVITY OF PARIEtal CORTICAL AREA 7B NEURONES DURING ACTIVE AND PASSIVE TOUCH. F. Tremblay*, S.A. Ageraatia-Béflanger, I. Zompa, C.E. Chapman. Centre de recherche en sciences neurologiques, Université de Montréal, Québec, Canada, H3C 3J7.

While it is known that lateral area 7 (7b) in the monkey contains cells with complex somatic and somatomotor response properties, the function of such cells, particularly those related to active hand movements, is at present unclear. In the present study, we were interested in determining if such cells play any role in a task requiring active scanning of textured surfaces. Recordings were performed in 3 monkeys (macaca mulatta) trained to discriminate textured surfaces (smooth vs smooth/rough) using either active touch (surface only explored with digit tips) or passive touch (surface passively dislocated under digit tips). The animal indicated the texture of the surface explored by pulling or pushing a lever with the opposite hand. Out of a total of 194 neurones, 45 had a somatic receptive field (RF; 32, somatic only; 13, somatic and visual) while 52 had no somatic RF (28 discharged specifically with Active Hand Movements, AHM units). While modulation was often observed in active (70%) or passive touch (50%), only a few cells signaled differences in texture (6%); such responses were not specific to the active task and no AHM units showed any relation to texture. The results suggest that neurones in area 7b do not play an important role in these relatively simple tactile discrimination, although a role in the analysis of more complex tactile stimuli cannot be discounted. (Supported by MRC-Canada and FRSQ.)

AUTOMATED DETECTION OF 2DG/IMMUNOSTAINED NEURONS IN BARREL CORTEX. L.S. Hibbard*, J.S. McCasland, and T.A. Woolsey, Departments of Neurology and Neurological Surgery, and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

With detailed immunohistochemical and metabolic maps of the barrel cortex, we expect to see a new picture of barrel function in the patterns of neuronal excitation/inhibition and energy use. These maps are montages of contiguous fields (125 µm, using a 40X objective with high NA) of immunostained sections and the corresponding autoradiograms, digitized under computer control. To detect neurones in GAD-stained sections, we correlate templates (averaged images of obvious GAD+ and GAD−cells) with the digitized fields. Potential cell locations in the field−images correspond to maxima in the 2-D correlation function $R = F(a)F(b)^*$, where $a$ and $b$ are the image and template, and $F$ is the Fourier transform operation. Detection is confirmed for features having high−valued normalized correlation coefficients with the templates. By inspection, false positive and false negative detections in GAD−stained sections are <2% of the detected cells. For each section, image collection (1280 digital fields, 320 Mbytes) and cell detection (>105 cells per image set) take 5 hours, running unattended under software control. This system can gather vast amounts of data quickly, and apply analyses to it which are both quantitative and exhaustive. By adjusting the templates, this method may be generalized for other histology preparations.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS: PSYCHOPHYSICS AND NETWORKS


We investigated the somatosensory perceptions evoked by stimulation of peri-rolandic as well as lateral parietal cortex in 50 epileptic patients undergoing a presurgical evaluation with intracerebral electrodes. Bipolar stimulation trains were delivered in an incremental sequence at medial and/or lateral contact pairs of stereotaxically-implanted multilead electrodes, while monitoring afterdischarge propagation with electrodes in frontal and temporal lobes. Lateral stimulation evoked: 1) contralateral sensations in Rolandic sites, 2) sensations on either or both sides in the opercular region, 3) mostly contralateral sensations in posterior parietal regions. Medially we evoked: 4) ipsilateral sensations in cingulate sulcus (around medial area 5), 5) contralateral sensations in posterior cingulate gyrus, and 6) bilateral sensations of changes in body position (levitation) in the region of the subparietal sulcus. These observations suggest the presence of distinct somatosensory regions in the human parietal cortex, some of which may show some correspondence to those identified in the macaque, such as PF, PECi, and PGm medially and PF and PFP laterally (Pandy & Seltzer, 1982).


645.12 CELLULAR MAPS OF METABOLIC ACTIVITY IN ANTIGENICALLY IDENTIFIED NEURONS: A 2-DEOXYGLUCOSE/IMMUNOSTAINING APPROACH TO BARREL FIELD CIRCUITRY. J.S. McCasland*, S. Kelmach and T.A. Woolsey, Department of Neurology and Neurosurgery, Washington University School of Medicine, Saint Louis, MO, 63110.

Local circuit axes in barrel cortex do not develop normally without input from the whiskers via the infraorbital nerve (PNAS 89:1832-1836). This and other evidence suggests that the pattern of connections within and between barrel columns is sculpted by activity, and we reason that the characteristic patterns of metabolic activation in normal behavior (Somat. Mot. Res. 8:111-116) reflect a dynamic equilibrium dictated by experiential manipulation. We have developed a new approach to examine cellular patterns of metabolic activation by combining a high resolution 2-deoxyglucose (2DG) technique, glutamate decarboxylase (GAD) immunostaining, and an automated detection algorithm (see companion abstract by Hibbard et al.). We compared 2DG labeling densities over GAD+ (presumably inhibitory) and GAD− (mostly spiny stellate and pyramidal) neurons of the awake behaving hamster. GAD+ neurons are heavily 2DG-labeled in every lamina of barrel cortex, more so in layer IV and less in layers II-III. Our data indicate that the ratio of 2DG labeling for GAD+ and GAD− neurons is approximately 2.0 in layer IV, 1.5 in layers V and VI, and 1.3 in superficial layers. These ratios are strongly correlated with overall labeling in their respective laminae, suggesting a dynamic matching between degree of local inhibition and local metabolic activation such that inhibitory influences become more predominant in heavily activated zones. Changes in the pattern of antigenically identified neuronal 2DG labeling patterns in the barrel field with different combinations of stimulated whiskers will be evaluated in behaving animals.

Supported by NIH grant NS17763.

645.13 OPTICAL INTRINSIC SIGNAL IMAGING OF SOMATOSENSORY CORTEX IN THE RODENT.

S. Narayan*, E.M. Szur, J. Burton and A.W. Tong, Laboratory of Neuro Imaging, Dept of Neurology, UCLA, Los Angeles CA 90024.

The detection of optical intrinsic signals enables the visualization of dynamic functional architecture in mammalian brain cortex. The response is known to be species and modality specific. Here we demonstrate the application of this method to functional mapping of whisker barrel cortex in the rat.

Male Sprague-Dawley rats were anesthetized (inhaled halothane, followed by urethane 1.5 g/kg) and stereotactically mounted. Prefabricated brass wres were cemented to the skull, centered over the postereor barrel subfield (MBPSF). The cortex was exposed, the well filled with artificial cerebrospinal fluid and sealed with a glass window. Contacted vibrators A4 to B4 were pulsed for 2 seconds at 4Hz. Synchronized digital images were acquired at 640 nm illumination with a slow scan cooled CCD camera. Stimulated images were divided by control images for timepoints post stimulation, and averaged over 20 to 100 trials. Optical intrinsic signals were observed as a focal decrease in reflectance (magnitude 10^-3 of control) over stereotaxically determined MBPSF cortex. The signal has two distinct spatiotemporal components. The first (diffuse) component begins 1.5-2.0 sec after stimulus onset, peaks at 2.5-3.0 secs, falls away by 4.5-5.0 secs, then exhibits an undershoot lasting approximately 3 secs. The second component (coincident with local vexcels) begins at 2.5-3.5 secs, peaks at 4.5-4.5 secs and dissipates by 5.5-5.6 secs.

This work demonstrates that optical intrinsic signals may be used to study functional cortical activity in the rat.
INTRINSIC SIGNAL OPTICAL IMAGING IN RAT SOMATOSENSORY CORTEX
J.L. Geiger, P. M. Gochin, P. Bodenbaugh, C. O. Gross\textsuperscript{a} and G. L. Gerstein. Dept. of Psychology, Princeton University, Princeton NJ 08544

The responses of somatosensory cortex to tactile stimulation of the forepaw were mapped by intrinsic signal optical imaging. The tips of digits 2 or 5 were repeatedly touched with mechanical tappers while CCD photographs were taken of S1 illuminated by an 800 nm light source. The resulting images showed two highlighted areas about 300 \textmu m in diameter and 500 \textmu m apart. Electrical recording in the areas highlighted during stimulation yielded receptive fields appropriate for the stimulated digit and not the other digit. Penetration between the highlighted areas had receptive fields on intervening digits. These results demonstrate that intrinsic signal optical images are obtainable in S1 and confirm the functional somatotopy previously reported using electrical recording. Furthermore, the short time required to produce the images and the spatial resolution suggest that optical recording could be used for the study of cortical reorganization in this brain region. Attempts to image activity from the hindpaw, and from adjacent digits of the forepaw yielded only weak and inconsistent signals. Furthermore, we have also been unable to detect acoustic activation of rat auditory cortex although we have replicated results of others in the cat (Frostig et al. 1995), demonstrating orientation stripes in their cortex. These observations may be encountered in the use of the optical imaging method.

SYNAPSES IN VENTROLATERAL POS (VLP) AND RETICULARIS PONTIS CAUDALIS (RPC) MEDIAN ELECTRICALLY EVOKED STARTLE. P.W. Franklin\textsuperscript{a} and J.S. Yeomans. Dept. of Psychology, University of Toronto, Mississauga, MSS 1A1, Canada.

Circuits for the acoustic startle reflex were analyzed by measuring hindlimb EMG latencies, and testing for collision in hindbrain sites in chloral hydrate anesthetized rats. In medulla and caudal RPC sites, latencies recorded in posterior biceps femoris had a mean of 4.5 ms and varied by less than 0.2 ms, when two 0.1 ms pulses were delivered at a 1.0 ms interpulse interval at current 2.5 times threshold. Latencies in other hindlimb muscles were also reliable, but latencies between muscles varied by up to 2.5 ms. The shortest observed latency following medulla stimulation was 3.6 ms, in posterior biceps femoris. As electrodes moved to rostral RPC and VLP, latencies increased by 0.3-0.4 ms. Collision tests between medulla and rostral RPC or VLP resulted in asymmetry with 0.3-0.4 ms collision intervals, suggesting a monosynaptic connection in RPC (Hempel et al., 1990). Between rostral RPC and VLP sites, or between medulla and caudal RPC sites, symmetric collision was observed suggesting connections by axons. Another increase in latency of 0.3-0.4 ms was observed in rostral VLP sites, along with a second increase in collision interval of 0.3-0.4 ms, suggesting that a second monosynaptic connection in rostral VLP near the periolivary area mediates the startle reflex. (Supported by NSERC grant A7077 to J.S. Yeomans.)
646.5 CONDITIONAL FEEDBACK OF HUMAN MUSCLE SPINDLE AFFERENTS. S.E. Grill, A. Blavo, W. Z. Rymer* Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892; Univ Göteborg, Gothenburg, Sweden; IRC of Medicine, Gainesville, FL 32610.

The presence of parallel intralimus/extralimus activation may conceivably enable spindle afferents to function as a model reference system. If such a system were intact, spindle afferent discharge would remain constant during commanded movements; changes in the feedback signal would be conditional upon whether there are situations in which spindle afferents display constancy of discharge during movement by recording microneurographically from single human muscle spindle afferents from finger extension muscles. Here we evaluated whether discharge variations in short latency movements against various-sized isotonic loads ranging from 2 to 17% of maximum voluntary torque (MVT) were affected by task demands and that the discharge rate increased or decreased at the onset of the shortening movement in a step-like fashion. The rate changed little during the subsequent shortening movement (see fig). This was true for 4 of 5 afferents studied, for at least one isotonic load level (p 0.05). It was not true, however, for all loads studied for each afferent. These findings suggest that conditional feedback may be operational during short, voluntary finger movements but only under certain loading conditions.

Work performed at Dept Physiology, Univ Umeå, Umeå, Sweden

646.6 IMMOBILIZATION HAS A MINIMAL EFFECT ON MUSCLE AFFERENT RESPONSES TO STRETCH IN A CAT HINDLIMB MUSCLE. M.A. Nordström,1,2,3 R.M. Enoka4,5, R.M. Fleischer,1 and D.G. Stuart1,2. Dept. of Physiology and Exercise & Sport Sciences, Univ. of Arizona Tucson AZ 85724, and Dept. of Physiology,2 University of Adelaide, Australia.

We studied muscle spindle and tendon organ afferents in cat tibialis posterior muscle after six weeks of right-hindlimb immobilization. The effects of this protocol on muscle force and fiber cross-sectional area have been reported previously (Nordström et al., Neurosci. Lett. 17, 648, 1991; Calleter et al., Neurosci. Abstr., 17, 649, 1991). Seventy-eight afferents (21 la, 34 sp II, 23 Ib) were used to monitor reflective stretch during the stretch were 144% and 135% of control, respectively; and 2) increased static length sensitivity of sp II afferents (the static length was 173% of control) and an increase in one index of dynamic sensitivity (148% of control). In all cases, significant differences were seen only with stretch from 20 to 60 ms after entry. For la and sp II afferents there were no significant immobilization effects in any variable. In summary, the effects of immobilization on muscle proprioceptive afferents were relatively minor, and would not appear to be a confounding problem for recovery other than the weakness associated with muscle atrophy. Supported by USPHS grants NS 20544, HL 07249, NS 25077, NS 0709 and RR 05675. M.A. was a C.J. Martin Fellow of the NH&MRC of Australia.

646.7 REFLEX RESPONSES TO UNEXPECTED LOSS OF FOOT SUPPORT IN INTACT AND CHRONIC SPINAL CATS K.G. Pearson*, M. Goraisin, G. Hofert and A. Prochazka Div. Neuroscience., University of Alberta, Edmonton, AB, CANADA

Adaptive responses to loss of ground contact during gait were compared in intact and spinalized cats. In normal cats, a walkway equipped with a trap door that opened just prior to hindlimb contact was used. Behavioral responses to this perturbation depended greatly on prior experience and the speed of locomotion. For example, in naive cats, the leg entering the hole remained extended for approximately 200 ms after entry whereas after a few trials, extension was shortened to 80 ms and the cat quickly flexed its leg out of the hole. When the cat trotted across the platform, the contralateral hindlimb commenced its swing phase at the time the ipsilateral foot entered the hole and as a result, led to a prolonged extension into the hole. Spinalized cats whose forelimbs were supported and hindlimbs walking on a treadmill with a hole cut into one side of the belt exhibited responses to loss of ground contact similar to naive, intact cats. During spontaneous walking, latencies to initiation of flexion were >200 ms and these responses did not change with repeated exposure to the hole. With concomitant perineal stimulation, however, the latencies to initiation of flexion were significantly reduced, but not to the minimal values seen after a few stimuli presentations in intact cats. This indicates that the fast flexion responses in experienced intact animals are supraspinally initiated and dependent on anticipatory set.

Funded by Canadian MRC & NCE & Alberta Heritage Foundation.

646.9 QUANTITATIVE ASSESSMENT OF INTERSEGMENTAL REFLEXES BETWEEN THE TAIL AND HINDLIMBS IN THE AMARE CAT. M. Friedman*, C. J. Vierck, Jr., and L. A. Ritz. Departments of Neuroscience and Neurosurgery, University of Florida College of Medicine, Gainesville, FL 32610.

We have previously investigated the hyperreflexia observed in segmental reflexes of the tail of cats after a chronic sacrocaudal transaction (Friedman et al., 1990, 1991). Here we report on observations of intersegmental modulation of reflexes of the tail and hindlimb. Normal cats were conditioned to receive innocuous electrocutaneous stimulation to the tail and then the hindlimb (or vice-versa). Interstimulus intervals (ISIs) in the condition-test paradigm were 20, 50, 100, 250, 500, and 1000 msec. Quantitative assessments of reflex force were performed by tethering the tail and hindlimbs to strain gauges. At short ISIs we observed facilitation of the reflex; however, at intermediate intervals there was a decrease in amplitude of the test reflex. In addition, conditioning stimulation affected the amplitude of hindlimb reflexes greater than that of the tail. With this approach, we will be able to characterize segmental intersegmental reflexes prior to and after specific lesions of spinal cord. Supported by grant NS27511.

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646.10 IPSI- AND CONTRALATERAL AFFERENT INTERACTIONS IN CAT SACROCAUDAL MOTEUNEURONS. LOUIS A. Ritz2 and ROBERT M. FRIEDMAN. DEPARTMENTS OF NEUROSCIENCE AND NEUROSURGERY, UNIVERSITY OF FLORIDA, GAINESVILLE, FL 32610.

Anatomical evidence from our laboratory has demonstrated that tail Ia fibers project bilaterally and that sacrocaudal motoneurons have bilateral dendritic trees. Sacrocaudal motoneurons, innervating tail muscles, might receive monosynaptic input from contralateral dorsal roots. Intracellular recording techniques were used to investigate contralateral influences on sacrocaudal motoneurons.

In most cases, contralateral input was weakly excitatory, at a latency of 0.7-1 msec longer than that of ipsilateral input. When contralateral and ipsilateral inputs were combined, there was usually a late augmentation of the compound EPSP, compared to that produced by ipsilateral input alone or occasionally there was complete inhibition of the compound EPSP at high stimulus rates. In other cases, the contralateral input inhibited ipsilateral responses at 5-10 msec and longer. We have thus far found no conclusive physiological evidence for monosynaptic input from contralateral dorsal roots to sacrocaudal motoneurons. Supported by NS27511.
646.11
INHIBITORY INFLUENCES OF PERIPHERAL C FIBERS ON THE FLEXION WITHDRAWAL REFLEX IN SPINAL CATS. J.A. McMILLAN, J.D. Long and J.J. Forsythe. National Naval Medical Center and WAMI Program, Montana State Univ., Bozeman, MT 59717

We reported earlier (McMillan et al., 1989, Soc. Neurosci., Abs. 15-918) that the peripheral C fibers contribute tonically to the flexion reflex in spinal cats. We report here C fibers can in fact inhibit the reflex.

Experiments were performed on cats initially decerebrated under ketamine anesthesia. The SF, evoked by stimulating the left sciatic nerve at 10 Hz, was monitored by recording isometric tension from the left semitendinosis. To evaluate influence of cunylated vs unmyelinated fibers we added C fibers to, or removing them from,Fore gressing the intensity of the stimuli.

C fibers were consistently excitatory on the SF in the decerebrate state. After cutting the spinal cord (T2-L4), the C fibers showed either an initial excitatory and subsequent inhibitory effect or just a pure inhibitory effect. In only 1 of 10 cats did C fibers have a pure excitatory effect in the decerebrate state.

These observations support a population of interneurons which are inhibitory on the SF. We propose a model in which these neurons (i) receive more excitatory inputs from the C unmyelinated afferents and (ii) more strongly inhibited by descending inputs than are the interneurons which are inhibitory on the SF. (Supported by NHP 86-1948, NSF EPSCOR RII-3921978 and NH 585956GS0198-0109.)

646.12
THE STRENGTH OF RECURRENT INHIBITION BETWEEN LATERAL AND MEDIAL GASTROCNEMIUS MOTONEURONES IN THE CAT. M.L. McCurdy* and T.M. Hamm. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013

The strength of recurrent inhibition is reported to be greatest between motor pools that innervate muscles with common periods of activity, within homonymous motoneuron pools, and between motor pools of muscles in close proximity (Burke et al., 1971; Fyffe, 1991). We thought it important to evaluate the strength of recurrent inhibition of spinal motoneurons (Smith et al., 1967; Rall, 1964; Koch et al., 1977). CAT SPINAL MOTONEURONES. T.M. Hamm* and M.L. McCurdy. Div. of Medical Informatics and Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Both the magnitude of the conductance changes associated with repetitive stimulation of muscle nerves. The preparation used has been accomplished with a single glass microelectrode using a discontinuous current clamp. Intracellular recordings were made of the voltage response to the injection of a mixture of sinusoidal currents (2.5-300 Hz), which provides information on the location of the conductance change (Fox, 1985). Current injection and voltage recording were accomplished with a single glass microelectrode using a discontinuous current clamp. Sets of voltage and current records were then subjected to Fourier analysis to determine power spectra. Our preliminary data show a small decrease in the voltage response in trials with recurrent inhibition, indicating an increased conductance. The change in the voltage response is greater at lower frequencies (<300 Hz), consistent with the dendritic location of the synapses in this pathway. These results indicate that the inhibitory effects of recurrent inhibition are attributable to conductance changes in addition to hyperpolarization. Supported by NS12454, NS07309 and GM08400.

646.13
CONDUCTANCE CHANGES PRODUCED BY RECURRENT INHIBITION IN CAT SPINAL MOTONEURONES. T.M. Hamm* and M.L. McCurdy. Div. of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

It is often suggested that Homonymous pairs of motoneuron pools innervating the muscle anterior-middle biceps femoris had significantly greater RIPSPs than homonymous pairs of MG, but not LG, motoneurons. This result suggests that the strength of recurrent inhibition in homonymous pools is not homogeneous for all species of motoneurons. Supported by NS22454, NS07309 and GM08400.

646.14
CAT SPINAL MOTONEURONES. T.M. Hamm* and M.L. McCurdy. Div. of Medical Informatics and Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

In 1 experimental cat model, we have previously demonstrated that the natural tactile sensory information in the same manner as shown in the cat experiments.

The strength of recurrent inhibition in homonymous pools is not homogeneous for all species of motoneurons. Supported by NS22454, NS07309 and GM08400.
CONTROL OF POSTURE AND MOVEMENT: ARM MOVEMENT II

647.1 MOVEMENTS TO APPARENT AND TRUE MOTION TARGETS. N.E. Port*, G. Pellizier, and A.P. Georgopoulos. Brain Sciences Center, VAMC, Minneapolis, MN 55417; and The Graduate Program in Neuroscience, Univ. of Minnesota, Minneapolis, MN, 55455.

Apparent motion is a well known perceptual phenomenon in which visual objects appear to be moving when physically they are a series of still frames. Apparent and real motion were used to assess sensory-motor performance in intercepting targets. Normal human subjects were asked to intercept targets traveling in a square by moving a pointer from the center point to the midpoint of a target's path. In the true motion condition, subjects intercepted a target moving smoothly in a square around the center point. In the apparent motion condition, the target appeared only at the corner points of the square, but the subjects were required to intercept the midpoint of the "apparent moving" target. Target velocities were varied across blocks ranging from 1 deg/s to 9 deg/s.

Subjects successfully intercepted the target in both conditions. Interception of the apparent moving target was slightly but systematically worse than that of the real target. The error in interception increased in an approximately linear fashion with velocity for both types of motion. A similar increase was observed in the variance of the error. These results show that the motor system can successfully utilize information from apparent motion and that visual-motor performance is very similar under conditions of real or apparent motion. (Supported by 1-PSMH48185-01).

647.2 MOVING TO THE SYMMETRICAL DIRECTION FROM A VISUAL STIMULUS. G. Pellizier1,4, G. Lome3, and A.P. Georgopoulos3. Brain Sciences Center, VAMC, Minneapolis, MN 55417; Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455; and 3Lab. de Physiologie Neurosensorielle, CNRS, 75270 Paris, France.

Bilateral symmetry was used to study perceptual mechanisms since the pioneer work of Mach nearly a century ago. Basically, the perception of bilateral symmetry is more salient when the axis of symmetry is vertical, i.e., when the axis is horizontal, and the least salient when the axis is oblique. We studied the perception of symmetry when the motor system is involved. For this purpose, normal human subjects were tested in a series of experiments in which the stimuli consisted of a red line (axis of symmetry) and a black line starting from the center of the red line and at an angle from it. The orientation of the axis and the angle of the black line were varied. Subjects were asked to make an arm movement (M) in the symmetric direction from the black line (B) relatively to the axis of symmetry (A). We measured the reaction time (RT) and the spatial accuracy. The perception of symmetry was tested in two additional psychophysical tasks. We found that movement RT increased with the angle of the black line. Moreover, the rate of increase varied with the orientation of the axis of symmetry, and was lowest when the axis was vertical. These results suggest that the intended movement direction is mentally rotated from the axis of symmetry toward the correct direction, and that the rate of rotation depends on the orientation of the axis of symmetry. Similar effects were obtained in the perceptual tasks. (Supported by NIH and HFSF).


We have previously shown that deafferentation disrupts the temporal coordination of multijoint arm movements (Neurosci. Abs. 17:553). We now ask whether this disruption results from failure to control joint interaction torques that develop from motion of mechanically coupled limb segments. We studied horizontal movements in normal subjects and patients with upper limb paralysis. Subjects were to trace a series of straight paths in different directions from a central starting position on a digitizing tablet. They were to make single overlapping outward and inward movements reversing direction without stopping. Elbow and shoulder angular displacement and EMGs of elbow flexors and extensors were recorded: hand paths and joint torques were calculated. Controls produced straight paths, with bell-shaped elbow and shoulder joint distributions which reversed direction simultaneously. The patterns of elbow muscle activity varied systematically with movement direction, acting to accelerate the limb or to counteract the interaction torques produced by motion of the upper arm. The paths produced by deafferented patients were severely distorted; joint displacements were asymmetric and interaction torques at each joint were temporally decoupled. The distortions varied with the direction of movement and reflected the magnitudes of the interaction torques at each joint. Muscle activation patterns did not have a consistent temporal structure and were not matched to the differences in interaction torques produced by movement. Proprioceptive input is therefore essential in the control of interaction torques which arise during multijoint movements. Supported by NS 227715, NS 25149, and a VA Merit Review.

647.4 SELECTIVE EFFECTS OF MUSCIMOL MICROINJECTIONS INTO CEREBELLAR NUCLEI IN CATS PERFORMING BOTH A Locomotor AND A REACHING TASK. M.S. Milak*, V. Vrba, V. Galli, Y. Bracha, P. Kolb, J.D. McAllduff, J.R. Bloedel. Barrow Neurological Institute, Phoenix, AZ 85013.

These experiments were designed to test the hypothesis that the individual cerebellar nuclei play different roles in regulating the performance of two different tasks. Each animal was trained to walk on a treadmill while avoiding a bar injected into each swing phase and to perform a reaching task in which a manipulandum was moved through a grooved plexiglass template in a sequence of 2 straight movements. The effects of muscimol injections into individual cerebellar nuclei ipsilateral to the performing extremity were assessed during both tasks. The EMG was recorded from forelimb muscles, and the kinematics of the same extremity was assessed using an active LED system.

Microinjections in the dentate and fastigial nuclei affected the coordination of the locomotor cycle. Injections in the anterior interposed nucleus (AIN) resulted in an inability of the animal to perform a flexion adequate to avoid the bar, thereby forcing the animal to adopt a different strategy to avoid the perturbation. In the reaching task, dentate injections resulted in a distinct tremor before contact with the manipulandum and an apparent difficulty in smoothly directing the sequence of movements. Injections in the AIN caused marked ataxia before bar contact as well as changes in posture before the initial reach was performed. Even though movements were impaired following these injections, the movement sequences could still be performed and actually improved with practice. NIH Grant RO1 NS21958.
647.5
MUSCLE ACTIVATION WAVEFORMS DURING ARM MOVEMENTS OF VARYING DISTANCE: C. Bacejo*, J.F. Sceatling, and M. Flanders. Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

We have previously shown that during arm movements of fixed distance and varying speed, the amplitude scaling of muscle activation waveforms can be described by a summation of two components: a phasic component that scales with speed (or movement time) and a tonic component that remains relatively constant. In the present study, activation waveforms from 7-9 shoulder and/or elbow muscles were examined in 15 moderately impaired subjects. Speeds varied continuously over the range of distances and speed to stationary targets aligned in the sagittal plane. All movements were from a fixed initial position. Movement times ranged from 300-1500 ms.

Observation of the electromyographic (EMG) waveforms revealed that the amplitude of the tonic component was dependent upon the distance of the movement. The amplitude of the phasic component was related more to movement time than to the speed of movement: shorter movement times were associated with more phasic EMG waveforms.

647.6
CHARACTERISTICS OF ARM MOVEMENTS IN NORMAL SUBJECTS AND SUBJECTS WITH UNILATERAL BRAIN LESION: C.A. Gianulli*, P. Gergory, J.L. Plow. Motion Analysis Lab, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7153

The purpose of this study was to characterize the motor control of arm movement during a tapping task in normal subjects and subjects with unilateral brain lesion. A method for analysis was developed to characterize differences between the groups. Data were analyzed from five normal subjects and five subjects with unilateral brain lesion. Subjects were instructed to catch and match target velocity with a stylus. Six trials were videotaped and three trials were digitized at 60 Hz. Variables calculated from the digitized data of a marker on the stylus were: number of tap cycles, mean latency, event, and cycle time, vertical displacement and velocity. An FFT was performed for each trial, and vertical velocity/position phase planes were calculated. Analysis of normal data revealed consistent cycle, event, and latent periods within and across trials with the greatest variability in the latent periods, and for the non-dominant arm. Latent period doublings were more common in the non-dominant arm than the dominant arm, and observed more frequently within the first few cycles of the trial. Analysis of the stroke amplitude revealed increased variability in all variables across trials, increased cycle periods, decreased mean velocity, a broader distribution of the frequencies in the power spectrum, and an increased range of vertical amplitude for the hemiparetic limb compared to the contralateral limb. These analyses appear to be sensitive indicators of motor control differences between limbs and for deficits in subjects with unilateral brain lesion.

647.7
KINETIC ANALYSIS OF PLANAR TWO-JOINT ARM MOVEMENTS IN HEMIPARETIC STROKE: R.F. Beer*, T.P. Dauwalder, and W.R. Rumer, Dept. of Biomedical Engineering and Physiology, Northwestern University, Chicago, IL 60611

There have been relatively few quantitative studies of the disturbed motor performance associated with hemiparetic stroke. Accordingly, we have conducted a preliminary investigation of the kinematics associated with performance of planar two-joint arm movements by a number of moderately impaired subjects.

Subjects were seated in a chair in front of a horizontal surface upon which thirteen targets were arranged in an unscalable distance and one central marker which served as the beginning point for all movements. A set of straps were used to immobilize the torso and shoulder girdle while the wrist and finger joints were immobilized using a fiberglass cast. Subjects were instructed to move as fast as possible from the central starting point to a designated target without regard to movement accuracy. The OPTOTRAK/3010 motion analysis system was used to track the position of an IRED located distally on the hand. EMGs were recorded from the major flexors and extensors of the elbow and shoulder using surface electrodes. Two sets of experiments were conducted. In the first set, subjects were required to actively generate the anti-gravity torques necessary to maintain the arm in a horizontal plane. In a second set of experiments the protocol was repeated with the upper limb passively supported.

For unsupported movements, trajectories were most disturbed in directions involving shoulder flexion and elbow extension, and hence, increased gravitational torques at the shoulder. Angular velocity profiles were distinctly asymmetric, with a prolonged deceleration phase. Most striking was the positive effect provided by arm support. Trajectories became nearly linear, velocity profiles bell-shaped, with as much as a 30% increase in peak joint velocities. Our results suggest that moderately impaired subjects retained a significant residual capacity to plan and execute goal-directed movements. We are currently examining various hypotheses that may explain the radical deterioration in movements requiring stabilization against gravitational loads.

This work was supported by NS 19331 to WZR.

647.8
THE CONTRIBUTION OF VISUAL AND EYE MOTION SIGNALS IN DIRECTING THE ARM TO MOVING TARGETS: P. van Donkelaar, R.G. Lee and R.S. Gellman, Dept. of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Normal human subjects were required to track a moving target with their extended index finger either (i) with full vision of the arm and unrestricted movement of the eyes, (ii) without vision of the arm, or (iii) while fixating a stationary target. The subject initially pointed at the target when it appeared at its starting position. After a variable delay the target started moving to the right and the subject was required to catch up to and follow it as accurately as possible. In each condition target velocity was varied randomly from trial to trial. Under normal conditions subjects were able to catch up to the target and match its velocity. Without vision of the arm subjects did not catch up to the target, but did match target velocity. However, when the arm was visible for the first 400-600 ms of the response the accuracy increased.

When subjects were required to fixate they caught up to the target, but then produced a steady-state hand velocity which was greater than target velocity. This is consistent with studies which demonstrate that target velocity is perceived as greater during fixation. Together these results suggest that extraretinal signals concerned with eye movement are needed to produce an appropriate hand velocity. However, one needs to see the position of the limb relative to the target to produce an appropriate hand position.

647.9

During naturally executed movements, the radius of curvature of the trajectory (T) and the tangential velocity are related by a power law (V_T ~ r^a). How can one ask if the recognition of curvilinear T can be altered by imposing different velocity profiles (V_p)? A robot arm was used to transport repeatedly the hand along a circular arc centered and aligned with a horizontal plane (A) at the target. Data from 16 subjects show that V_T ~ r^{0.44} in phase 1; and V_T ~ r^{0.23} in phase 2. Some subjects could declare that they had found the right strategy after one or only a few trials, before they had achieved successful scaling.

The paradigm required subjects to guide a display cursor from a start-box to a target-box by moving a pen on a digitizing board. Hand positions modulated cursor velocities ('rate control'). This correct strategy was to start and stop the cursor with two oppositely directed hand movements, and correct tactics required their amplitudes to be close enough to each other to stop the cursor in target, within the time set by the paradigm. Sessions of 200 trials took 25 min. Phase 1 began with naïvely ended with strategy selection (measured as task performance with no more than 2 consecutive non-strategy trials), attaining different subjects in up to 111 trials. End of phase 1 triggered phase 2, the highly visible movement rescaling, that ended with stable ratios of stop and start move amplitudes (ratios sufficiently close to 1 in 9 consecutive trials). Rescaling phase 2 was completed by subjects using the correct strategy in 7 to 14 trials (in a total of 7-25 trials); and by three subjects with the strategy in 34 to 56 trials (in 44-61 total). The number of trials to attain stable amplitudes for the correct pattern strategy is in the range of trials reported for adaptive scaling attributed to cerebellar function.

This simple kind of test with easy read-out could be used for recording and imaging brain activity during visuomotor learning.
647.11
COORDINATION OF SINGLE AND DOUBLE JOINT MUSCLES OF THE ELBOW. L.E. Serio* and D.J. Ostry. McGill University, Montreal, PQ, Canada H3A IB1

In previous work with A.G. Feldman and J.R. Flanagan we have suggested that central commands specify the equilibrium point in multi-joint movements by controlling many muscles in concert. In particular, for both arm and jaw movements we have proposed central commands which control motion in different degrees of freedom as well as the level of coactivation without motion. The basic problem is that since muscles do not - in general - act in individual degrees of freedom central commands must be coordinated to produce motions in separate degrees of freedom. In this paper we report an empirical study of the coordination of central control signals necessary to produce movements about the elbow involving flexion alone, supination alone, and combinations of the two. The work was carried out by examining the kinematic and electromyographic patterns associated with 3D arm movements. EMG was recorded from eight single and double joint elbow muscles as subjects made pronation / supination and flexion / extension movements of different magnitudes. Shoulder elevation and orientation were also varied. Kinematic patterns were recorded using WATSMART. The data presentation focuses on the relationships between kinematics and EMG activity associated with motion in more than one degree of freedom.

647.12

When humans point without seeing their arm, successive errors tend to accumulate, which suggests that pointing is amplitude- rather than position-controlled1. We now compare trends for error accumulation and for error correction for movements with varying directions.

Six humans pointed, without seeing their arm, at mirror-viewed targets. The targets appeared sequentially in a frontal plane and required direction changes between successive movements of 0, 45, 90, 135 or 180 deg. Pointing accuracy was registered by the Watsmart® system, and we calculated the linear regression between successive pointing errors. Positive correlation indicated error accumulation, the regression slope S indicated the relative roles of accumulation (S=1) and correction (S=0). We found that R>0 throughout the sequences (mean: 0.53, p<0.01), and that R was significantly smaller for movements preceded by a direction change of more than 45 deg (0.37 versus 0.71, p<0.01). We also found that S<0 (mean: 0.60, p<0.01) and that S depended on direction changes in such the same way as R (0.42 versus 0.75, p<0.01). We conclude that error accumulation persists, albeit reduced, across changes of movement direction, and that a complementary trend for error correction exists. Correction is probably due to proprioceptive feedback since it is absent in deafferented subjects2.


647.13
TIMING AND AMPLITUDE CONTROL OF REACHING AND GRASPING. P. Cordo* and M. Schieppati. Institute of Human Physiology II, University of Milan, Italy

Reaching and grasping is a multijoint movement involving a sequence of actions that include transporting the arm, orienting the hand, and opening and closing the fingers. This movement sequence is controlled by many of the muscles of the shoulder, arm and hand. The purpose of the experiment reported here was to determine what principles underlie the coordination of such activity in these muscles.

Normal human subjects, seated at a table, reached for an L-shaped handle located at 1 or 3 heights, with 1 or 3 orientations with respect to the vertical plane. Subjects grasped the handle with one of these 9 combinations of height and hand orientation in contiguous blocks of 10 repetitions. The movement time to handle was constrained by playing a series of tones through headphones. We recorded the activity of up to 7 muscles with surface and wire electrodes: upper trapezius, anterior deltoid, biceps brachii, supinator, flexor digitorum communis, and extensor digitorum superficialis.

We made the following observations with these reaching and grasping movements: 1) different muscles are involved in the control of reaching height, hand orientation, and hand opening and closing, but some muscles can control more than one of these kinematic variables, 2) the control of a given variable can be via the onset timing of activity or via the amplitude of activity, and 3) changes in the level of muscle activity occur at discrete, periodic times during the movement.

647.14

Muscle tendon vibration is known to excite muscle spindles, the primary source of information for the perception of limb position in the absence of vision. Vibration of the biceps of a stationary or slowly moving arm causes the perception of elbow position to be biased towards extension. During slow tracking tasks undue flexion of the elbow results. Contrarily, in fast step-tracking tasks correct positioning is achieved despite vibration, even if the arm was unduly flexed before the step as a result of matching arm and initial target position. We now asked if we could make subjects perform fast goal-directed movements when they thought their arm was already at the target position. Four subjects tracked slowly moving targets. A mirror covered the arm. The image of a light above the mirror formed the target such that the hand and the target moved in the same plane below the mirror. Biceps vibration induced undue flexion. At times subjects were asked to grab the target with their hand. All subjects then performed fast and often substantial elbow extension movements reaching the target and, when asked about this, claimed they had not made any such fast movements. Electromyograms recorded from biceps and triceps show that the muscles were activated if the subject were performing a voluntary fast movement. We conclude that the programming of fast movements does not depend on the conscious decision to perform such a movement, and that the performance of a fast movement in itself does not give rise to the perception of movement.

647.15
A COMPUTATIONAL MODEL FOR OPTIMAL PLANNING AND CONTROL OF LIMB MOVEMENTS. N. Last* and P. E. Cargo. Applied Neural Control Lab, Dept. of Biomedical Engineering, Case Western Reserve Univ., Cleveland, OH 44106, and Center for Biomedical Engineering, University of Kentucky, Lexington, KY 40506

In this abstract we present a computational model for generating limb movement trajectory, muscle control signals, and the associated joint stiffness and equilibrium states. The model includes a central planning algorithm derived from a minimum time criterion, a spinal circuit integrating reciprocal inhibition, and the associated joint stiffness and equilibrium states. The model is able to reconcile many lines of experimental findings about single joint voluntary movements. It can also be extended to multi-joint movements.

Acknowledgments: this project is supported by the Spinal Cord Research Foundation of PVA and NIH.
648.1 HIZELINE PLANE ATTRACTION IN THE LOCOMOTION OF NORMAL AND DOPAMINE SIMULATED, TREATED RATS. L. Golani, R. Einat and P. Teitelbaum. Dept. of Zoology, Tel Aviv University, Ramat Aviv, Israel and Dept. of Psychology, University of California, Riverside, Florida, U.S.A.

In our work we search for "natural" frames of reference and collective variables, presumably used by the brain in the coordination of movement. The rat's midline plane, which divides body-related space into two symmetrical hemispheres, was used as a reference plane for the recording of five degrees of locomotor behavior. A comparison of the behavior of rats with saline and three dopamine stimulants revealed three distinct locomotor profiles which differed in the strength of attraction of the anterior body parts to the midline plane. With saline and apomorphine rats showed a significant attraction to a plane parallel to the head to this plane. Quinpirole greatly enhanced and apomorphine first enhanced and then greatly reduced this attraction. This suggests that the midline plane is used by rats as a reference plane in the coordination of trunk movements. The head's angular displacement from this plane could be a collective variable used in the coordination of locomotor behavior.

648.2 RECIPROCAL INHIBITION (RI) DURING VOLUNTARY DORSIFLEXION OF THE ANKLE: A LINEAR RELATIONSHIP. J.E. Tremblay* Ottawa Univ., 2) School of Medicine, University of Miami, 3) Department of Physiology and Life Sciences, University Lille 4000, Belgium.

RI is believed to play a crucial functional role in motor control with spinal inhibitory interneurons implicated. Tanaka (1974) found that RI was scarcely detectable in normal subjects at rest. He has shown that the reciprocal relationship of the triceps surae muscle becomes active during voluntary dorsiflexion of the ankle. The purpose of this study was to quantify the effect of voluntary isometric contraction in dorsiflexion on the H-soleus reflex. Twenty nine normal subjects (30 ± 8 years) were studied. Hoffman's H-reflexes in the soleus (S) was used to assess the changes in (RI) evaluated by electrical stimulation of the antagonist muscle (tibialis anterior) (TA) via the common peroneal nerve (CPN) using five condition levels; at rest, slight contraction, 1kg, 2kg and 4kg resistance in dorsiflexion. Subjects were given auditory and visual feedback of the EMG to help maintain a steady voluntary contraction of the TA muscle. The electromyograph was used to ensure the isometric condition. Stimulation of the CPN demonstrated a 9.2 ± 7% (P < 0.01) augmentation in RI in (S) at rest and 26.6 ± 17%, 46 ± 10%, 61% ± 10% and 58 ± 12.5% respectively for slight contraction, 1, 2, 4kg respectively. The relationship is linear until the regression curve is y = 1.2x + 11.96 (r = 0.94). A voluntary contraction of the TA muscle is one the best methods of RI reinforcement. The possible implications of Renshaw discharge and presynaptic inhibition will be discussed.

648.3 EFFECTS OF ANKLE EXTENSOR CONTRACTIONS ON CAT LUMBAR MOTONEURONS. L. Jami*, D. Zymicki, J. Lafleur and G. Bockstedt. Hospital for Sick Children, 1445, Côte-de-Février, Montreal, Quebec, Canada.

The effects of cutaneous afferent inputs generated by contractions of gastrocnemius medialis (GM) were recorded in ipsilateral lumbar motoneurones of chronically-amputated cats. Contractions were obtained by stimulating a cut branch of the nerve. In homonymous and synergistic motoneurones, GM contraction evoked a quick decline of group Ib input (1). Recordings of contraction-induced PAD in Ib terminals suggested that pre-synaptic inhibition of Ib effects accounts for the decline of auto-geneous Ib inhibition (2).

Declining inhibitions elicited by GM contractions were also observed in various other the motoneurones. On reperfusion of electrical stimulation of GM nerve, the strength of excitatory declining inhibitions similar to those evoked by GM contractions were 5.8-7.8 times group I Ib threshold, recruiting group II in addition to group I fibers. These observations suggest a significant contribution of group II afferents to the effects elicited by GM contractions in non-synergistic lumbar motoneurones. The mechanisms causing the decline of the contraction-induced effects might involve presynaptic inhibition of group II fibers.

In addition to tendon organs and spindle primaries, group II muscle afferents also contribute information about ankle extensor contractions, in the form of a negative feedback distributed to a variety of lumbar motoneurons and quickly filtered out, leaving motor units available for recruitment as may be required by motor coordination.


Records of EMG activity from locomoting spinal cats in our laboratory have indicated extended level of oscillations in amplitudes of EMG bursts (Lovely et al. Brain Res 514:206, 1990). In most cases, the bursts were divided into short 'packets' of activity separated by periods of silence. This contrasts with examples of EMG bursts in spinal cats from other laboratories where such clear oscillations were not apparent (Barbeau and Rossignol Brain Res 412:84, 1987; Forssberg et al. Brain Res 50:184, 1973).

The significant difference is that in our experiments the animals was that they were left to recover for one month before locomotor training was initiated. In more recent experiments, we have initiated training one week after spinalization and found much less oscillation in the amplitude of EMG bursts following spinalization. This observation suggests that the timing of therapeutic interventions in the treatment of spinal injuries may affect the outcome of those therapies. We have also analyzed the frequencies of EMG oscillations in control and spinal cats and in two instances compared tremor before and after spinalization in the same animal. The frequency of EMG oscillation in all spinal cats was approximately 25 Hz, compared to 50 Hz in controls. Similar results were found in the two cats studied before and after spinalization. Together these data suggest that descending pathways influence the spinal circuitry involved in generating tremor. (Supported by NIH Grant NS16333)

648.5 GAIT ASSISTANCE WITH FES CONTROLLED BY THE CUTANEOUS ENG. K. Kallieris*, M. Haugland and HI. Hoffman. 1) School of Kinesiology, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada, 2) Department of Medical Informatics and Image Analysis, Aalborg University, Aalborg, Denmark.

The activity of skin mechanoreceptors recorded by cuff electrodes implanted on sensory nerves can provide useful information on the forces applied on the skin. The effects of muscle activity on cutaneous mechanoreceptors were investigated in two cats in which the cutaneous electromyograph (EMG) was used for closed-loop control of functional electrical stimulation (FES) of paralyzed muscles in order to restore a precise grip reflex (Hoffman & Haugland, SN Abstr. 17:1031). We now present an application in the conscious, walking animal, in which the cutaneous ENG was used to control FES in order to assist gait.

The cat hindlimb served as a model to test this application. A tripolar nerve cuff was placed on the common peroneal nerve (CPN) using five condition levels; at rest, slight contraction, 1kg, 2kg and 4kg resistance in dorsiflexion. Stimulation of the CPN demonstrated a 9.2 ± 7% (P < 0.01) augmentation in RI in (S) at rest and 26.6 ± 17%, 46 ± 10%, 61% ± 10% and 58 ± 12.5% respectively for slight contraction, 1, 2, 4kg respectively. The relationship is linear until the regression curve is y = 1.2x + 11.96 (r = 0.94). A voluntary contraction of the TA muscle is one the best methods of RI reinforcement. The possible implications of Renshaw discharge and presynaptic inhibition will be discussed.

648.6 STRYCHNINE- INDUCED MODULATION OF EMG OF HINDLIMB FLEXORS AND EXTENDERS DURING STEPPING IN CHRONIC ADULT SPINAL CATS. P. de Guzman, J.A. Hodgson, B. Roy, C.P. de Leon, R. Roy and V.R. Edgerton. Department of Physiological Science and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Strychnine initiated full weight-bearing stepping in spinalized cats that were unable to walk and resulted in a more robust stepping pattern in those that were trained to walk (de Guzman et al. Soc Neurosci 17:1577, 1991). EMG activity of hindlimb flexors (semimembranosus, ilopsoas, tibialis anterior) and extensors (vastus lateralis, soleus-Sol, medial gastrocnemius) were examined in 4 adult spinalized cats (T12-T13) before and after the administration of 2 doses of strychnine (0.03 and 0.10 mg/kg, i.p.) to determine if the changes in the EMG pattern after spinalization in other spinal mechanisms. Two cats were trained to walk on the treadmill at varying speeds (0.2-1.0 m/s) and 2 were trained to stand. Training (30 minutes) was stopped after 1 week after spinalization. At 3 months post-spinalization the treadmill-trained cats walked at all speeds. Standing-trained cats were only able to walk for a few minutes after strychnine administration prior to falling. The standing-trained cats were able to walk after strychnine administration and higher doses increased the Sol mean amplitude in one of two cats. These data indicate a selective effect of strychnine on the amplitude of specific muscles and a maintenance of the EMG patterns in both flexors and extensors. The major influence of strychnine is on premotoneuronal networks that generate cyclic activity in the spinal cord rather than a direct effect on motoneuronal pools. (Supported by NIH Grant NS16333)
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Can cats as adults retain effective weight-supported stepping of their hindlimbs when properly trained over a period of weeks to step on a treadmill (Lovely et al. Brain Res. Neurosci. Mov. 10:181, 1985). Although it is known that the stepping patterns of these spinalized cats can accommodate varying speeds, loads and other sensory perturbations (Hopkins et al. J. Neurophysiol. 52:57, 1984), the extent to which supraspinal compensation to spinal cord injury has been defined. In the present study, EMG from selected flexors and extensors in 4 adult cats was recorded during bipedal stepping before and 1 to 3 months following spinalization (T12-T13). Two spinalized cats were trained to walk in a treadmill at speeds ranging from 0.2 to 1.0 m/sec and 2 cats were trained to stand. Each cat was trained for 30 minutes, 5 days/week. Training began 1 week after spinalization, at which time none of the cats was able to execute weight-supported stepping. The 2 cats trained to walk were able to walk with full weight support after 3 weeks of training. The standing-trained cats were unable to walk throughout the entire training period. Following an acute administration of atropine (0.03 mg/kg) 3 months after spinalization, however, these 2 cats could generate full weight-supported stepping (see accompanying poster, de Guzman et al.). Amplitudes within each burst of EMG activity were averaged over 5-15 consecutive step cycles. Double bursts per cycle were observed consistently in the semitendinosus and iliotibialis before spinalization while one of these bursts became much less prominent following spinalization. Packets of EMG bursts were observed frequently in the flexors, particularly in the tibialis anterior, after, but not before, spinalization. Generally, the EMG waveforms of extensors (vastus lateralis, soleus, medial gastrocnemius and gluteus medius) were similar pre- and post-surgical levels. These data suggest that the neural networks of the lumbar spinal cord that control the extensors can compensate more completely for the absence of supraspinal control than that of the flexors. Motor pools are more dependent on supraspinal control than extensors.

Supported by NIH Grant NS16333

648.8

GENERATING HUMAN LOCOMOTOR ACTIVITY PATTERNS USING AN ARTIFICIAL NEURAL NETWORK MODEL. S. D. Prestero* and A. E. Patla. Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

A neural network model was used to map out a relationship between desired locomotor trajectories and the necessary muscle activations. The proposed network incorporates a fully connected feed-forward model receiving twelve inputs, ten hidden units and four output units. The inputs include the vertical and horizontal displacement trajectories for the hip and toe, the hip and knee angles, and the time derivatives of these six inputs. The output consists of the muscle activation time histories of the major extensors and flexors of the lower limb - soleus, tibialis anterior, biceps femoris and rectus femoris. The bias values and connection weights of the network were learned using the back-propagation rule, using the NeuralWare software. A robust model must be able to control walking over uneven terrains. Therefore, data from subjects walking over obstacles of different heights were used to train and test the network. The test data set was presented at specific intervals during training to monitor the model's ability to generalize to novel data. The muscle activation patterns produced by the resulting model upon presentation of the training data closely matched those obtained experimentally. Presentation of the novel data also closely resembled the actual activation time histories. The performance of the model was quantified by calculating the correlation and root mean squared error between the actual and predicted curves. All muscles of the training data had correlations greater than 0.9 and RMS errors less than 0.1. Most of the muscles in the test data values had correlations greater than 0.8 and RMS errors less than 0.3.

648.9

DIFFERENTIAL EFFECTS IN PATHWAYS INTERCONNECTING KNEE AND ANKLE EXTENSORS IN MAN. B. Pelletier, R. Forget, D. Bourbonnais*. Centre de Recherche, Institut de Réadaptation de Montréal et l'École de réadaptation, Faculté de Médecine, Université de Montréal.

To study the specificity in spinal pathways interconnecting heteronymous muscles a conditioning H-reflex technique was used to investigate, at rest, the pathways interposed between the soleus (SOL) and the vasto-crusens (VC) muscles. Fourteen normal human subjects (27 ± 1.37 years) participated in the study. Two experimental conditions were investigated: (1) conditioning stimulation applied to the femoral nerve (FN) and a test stimulation applied to the posterior tibial nerve (PTN) and (2) conditioning stimulation applied to the PTN and a test stimulation applied to the FN. Under both experimental conditions the intensity of stimulation was adjusted so that conditioning stim. was at motor threshold and the test stim. at Hmax/2. The results for both experimental conditions was an initial short lasting facilitation (+5 ms) followed by a relatively long lasting (-50 ms) inhibition. However this inhibition was much stronger in the FN-SOL experimental condition. In this situation (FN-SOL) the Conditioning Test intervals (C-T int) had a significant effect on the conditioned H-reflex values (p = 0.001) and those conditioned values were found to be significantly different from their control values (p = 0.005). In the second experiment, although the C-T int had an effect on the conditioned values (p = 0.004) no difference between the conditioned and control values were demonstrated (p = 0.048). Different conditioning stimulation intensities of the FN were investigated at the C-T int where the strongest inhibition of the soleus muscle was present. The inhibition appeared with a stimulation strength of 1.0 X Reflex Threshold and increased in concordance with the amplitude of the H-reflex response in the conditioned muscle. Spinal mechanisms regulating inhibition between extensor muscles may facilitate the task of supraspinal structures in the regulation of heteronymous muscles important to maintain an erect posture in stance and gait. (R. Forget and D. Bourbonnais are funded by the FRQS)

648.10


Our previous studies have shown that, after spinal transection at T13 in cats, locomotor recovery with plantar foot placement and weight support of the hindquarters takes place between 2 to 3 weeks. We have also shown that injection of clonodermic drugs (clonidine and L-Dopa) together with perinatal stimulation on produce, in the first week post transection, a correlated locomotor pattern in the adult cat treadmill for several hours. We have taken advantage of this possibility to train cats on a treadmill after daily injection of clonidine (150 to 225 ug/kg i.p.) in the first post-transection week and study the effects of training (60-90 minutes) on the recovery of spontaneous locomotion. Electromyographic (EMG) activity synchronized to video images of the hindlimbs were recorded before and after clonidine injection. From the 3rd to the 9th day post transection, there was a marked increase in the duration of the step cycle accompanied by a gradually increasing increase in the duration of extensor EMG activity and a gradual decrease in the duration of flexor muscle activity. The increase in total angular excursion of the hip, knee and ankle joints was also evident. Concomitant changes in kinematics included a brisk transition from swing to stance, hyperextension of the ankles during late stance, and synchronous flexion of hip, knee, and ankle during early swing. It is noticeable that, from the 3rd to the 7th day, the effect of clonidine given on one day was not carried over the next day i.e. the spontaneous locomotion before clonidine injection was not improved. Therefore, we were able to elicit a locomotor pattern without clonidine injection by the 8th, the 9th and the 11th day respectively. These observations suggest that early locomotor training with clonidine accelerates the recovery of locomotion after spinalization. (Supported by the NCF, the MRCC and the FCAR).

648.11

EFFECTS OF MOVEMENT INITIATION CONDITIONS ON POSTURAL AND TASK EMG ACTIVITY DURING INITIATION OF RAPID SHOULDER MOVEMENTS. J. D. Lehman, D. D. Kikula and J. B. H. Shumway-Cook. Dept. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Studies were undertaken in 10 normal infants at ages 12, 18 and 24 weeks to investigate the characteristics of the early changes in the kinematics of stepping prior to the development of independent locomotion. Infants were supported over a treadmill moving at 0.06m/s and light emitting diodes were placed over crural markers on the lower limb. Three classes of lower limb movements were identified: alternate steps, hops and jumps. The active phase of alternate steps data were clearly different from SP and CA data. Ankle muscles were less involved in movement initiation in the RT condition, evidenced by later and less consistent burst onset. Activation patterns of other muscles were also different in RT vs SP and CA conditions, though in subject-specific ways. These data suggest that patterns of anticipatory postural activation can be highly sensitive to temporal constraints on movement initiation.

648.12


Studies were undertaken in 10 normal infants at ages 12, 18 and 24 weeks to investigate the characteristics of the early changes in the kinematics of stepping prior to the development of independent locomotion. Infants were supported over a treadmill moving at 0.06m/s and light emitting diodes were placed over crural markers on the lower limb. Three classes of lower limb movements were identified: alternate steps, hops and jumps. The active phase of alternate steps data were clearly different from SP and CA data. Ankle muscles were less involved in movement initiation in the RT condition, evidenced by later and less consistent burst onset. Activation patterns of other muscles were also different in RT vs SP and CA conditions, though in subject-specific ways. These data suggest that patterns of anticipatory postural activation can be highly sensitive to temporal constraints on movement initiation.

Supported by the NICHD.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
648.13 THE ROLE OF SOMATOSENSORY INFORMATION IN A CONSTRAINED LOCOMOTOR TASK. S.M. Henry, J.M. Held.* B.P. Vanijie and J. Wells. Dept. of Anatomy and Neurobiology, Univ. of Vermont College of Medicine, Burlington, VT 05405. Sensory information from the body is distributed and processed by different central nervous system structures which are blended in both sensory perception and motor activity. The present study examined the role of the dorsal column nuclei and the ventral-posterolateral (VPL) nucleus of the thalamus in a constrained locomotor task. Rats were trained to traverse an elevated 1\degree bar for a reward. The time it took to run across the bar was used as a measure of goal-directed behavior. The trajectories of the hindlimb during the swing cycle were quantified from videotape. The lesion groups were 1) right gracile nucleus, 2) bilateral gracile nucleus and left VPL, and 3) bilateral VPL. The animals were tested on the bar running task for 50 days post-lesion and filmed periodically. Two measures of loss and recovery of function were used: 1) the running time and 2) the movement topography of the hindlimb swing cycle. Only the bilateral VPL lesioned animals were significantly impaired in running times across week 1 post-lesion. However, all groups demonstrated an improvement in movement topography even on the day they returned to their pre-lesion run times. This improvement in movement was not recover. This result is similar to somatosensor cortex lesions and suggests that central processing of tactile information is important in constrained locomotion.

648.15 MUTABLE ACTIVATION OF BIFUNCTIONAL THIGH MUSCLES DURING FORWARD AND BACKWARD WALKING. C.A. Pratt, J.A. Buford and J.L. Smith, Dept. of Physiological Science, UCLA, Los Angeles, CA 90024-1566. In contrast to unifunctional muscles, the bifunctional semitendinosus (ST) has a distinctly different EMG pattern during the swing phase of forward (FWD) and backward (BWD) walking, suggesting that the activity of bifunctional muscles may be more mutable and adjusted by proprioceptive feedback to match different limb kinematics associated with the two forms of walking (Buford et al. J Neurophysiol. 64, 1990). To explore this possibility, the activity of two hip flexor-knee extensor muscles (anterior sartorius, SAS and rectus femoris, RF) and a hip flexor (flexor digitorum longus, FDL) were recorded along with limb kinematics (ciné film) in 3 cats trained to walk FWD and BWD on a motorized treadmill. Both of the hip flexor-knee extensor muscles had significantly different EMG patterns during BWD compared to FWD walking. The cats may have adjusted the activity among the muscles and appeared to reflect relative differences in their actions at the hip and knee. The shift occurs later at the knee during BWD swing (0.22 of the normalized step cycle; paw off at 0.0) than in FWD swing (0.10), but earlier at the hip (BWD = 0.12, FWD = 0.30). During FWD swing, activity in all three muscles ended at 0.29 and, thus, appeared to be related to their hip flexor action. In contrast, during BWD swing, SAS and RF activity (EMG offset = 0.04) was related to hip flexion, but SAa activity ended at 0.29 and was more related to knee flexion. FWD and BWD walking also differ in that the hip flexes during BWD stance. Typically, SAa was not active during either FWD or BWD stance. SAa and RF were both active during late FWD stance (0.68-0.64), but the two knee extensor were differentially activated during BWD stance: RF was active throughout stance extension (0.23-0.98), but SAa was active just briefly (0.26-0.47) around paw contact (0.35). In both FWD and BWD stance, SAa activity coincided with the reversal of hip extension to flexion. The temporal shifts in SAa activity were linked to the action and muscle activity at the toe (metatarsophalangeal joint) was not observed. Supported by NIH NS 19864.

648.16 THE EFFECTS OF TERRAIN DIFFICULTY ON CHARACTERISTICS OF VOLUNTARY VISUAL SAMPLING OF THE ENVIRONMENT DURING LOCOMOTION. P. C. Martin, H. Holmog & M. Prestin. Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Ontario, Canada, N2L 3G1. Young subjects (N=16) were instructed to walk over travel paths (9.1 m long) of varying degree, liquid crystal eyeglasses, and pressing a switch to make the glasses transparent when they needed to sample the environment. Their movement time (MT) & following visual sampling characteristics were recorded for visual samples (#SAM), total duration of visual samples (TDVIS); average (ADVIS) & variability of visual sample duration (SDVIS); average (AISI) & variability of intersample duration (ISI); and standard deviation of visual sample duration (ISI). A 2 (intervention) x 2 (order of walking path) x 2 (visual sample duration requirement) x 2 (one or two obstacles in path) repeated measures ANOVA revealed the following results. #SAM decreased in presence of obstacles compared with even step length (6.7 vs 7.7); TDVIS was higher for walking compared to straight path (3.66s vs 3.06s) & higher for uneven step length condition compared to even step length (3.46s vs 2.90s); SAS was higher when obstacles were present (0.35 vs 0.29). When the hole was included, in the travel path affecting the consequence of error in foot placement. SAa increased (8.3 vs 7.0) along with higher TDVIS (3.73s vs 2.85s); and AISI reduced (1.23s vs 1.36s). A significant test was constrained to place their feet on specific location, TDVIS (2.45s vs 0.88s) & #SAM (5 to 1) reduced dramatically. MT was similar for all conditions (9.36s). Inclusion of a large barrier in the walking path to influence preview region increased MT (11.9s vs 9.34s); AISI (1.76 vs 1.36s) & #SAM (0.57s vs 0.29s). These results provide insights into how intermittent visual sampling of the environment is normally used for navigation. (Supported by a grant from NSERC, Canada.)

648.17 EFFECTS OF TERRAIN DIFFICULTY ON CHARACTERISTICS OF VISUAL TASK-RELEVANT CORTICAL ACTIVITY DURING LOCOMOTION. R. Salmon*, A. Hoffman, B. Buschbeck & T. Drew. Dept. of Physiology, Université de Montréal, Canada. D3C 3F7. Experiments to determine the relative importance of different descending systems for the adaptive control of locomotion were performed in two cats which were chronically implanted with electrodes for recording electrical activity from muscles of the fore- and hindlimbs. Following control studies, a bilateral lesion of the spinal cord was made under general anesthesia at T13 which completely interrupted the dorsal columns (DC) and extended to differing degrees into the dorssal funiculus (DLF). In one cat, with a relatively small lesion of the DLF, locomotion recovered within one week, and the cat was readily able to adapt its locomotion to walk at different speeds and on different surfaces. In the other cat, with a larger DLF lesion, locomotion recovered more slowly over a period of 4-6 weeks, and even after this time there was often a tendency for the cat to place the hindpaw on its dorsal surface. Nevertheless, this cat could also walk at different speeds and on different surfaces. In both cats, the locomotion recovered to locomotion on inclined planes. Both cats, however, showed long-lasting deficits in their capacity to modify their hindlimb gait in order to step over obstacles attched to a treadmill or to locomotion on inclined planes. These preliminary results suggest that whereas pathways contained within the ventralateral and ventral spinal cord are sufficient for normal locomotion, pathways within the DC and/or DLF seem to be essential for anticipatory control. Supported by the NSERC, the NCE, and the Rick Hansen Man in Motion Legacy Fund.
649.1 DISCORDANT EXPRESSION OF ACETYLCHOLINESTERASE ACTIVITY AND mRNA LEVELS IN SINGLE NEUROMUSCULAR JUNCTIONS OF NORMAL MUSCLE. R.K. Lee, B.J. Jasmin and R.L. Rotundo*. Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33101.

Acetylcholinesterase (ACHE) is highly concentrated at the vertebrate neuromuscular synapse. Among the mechanisms that could account for this selective accumulation of ACHE molecules is localized transcription of the ACHE gene in post synaptic sarcoplasmic nuclei. One prediction of this model is that ACHE enzyme at the neuromuscular junction (NMJ) would mirror the levels of ACHE transcripts. To test this, we used a PCR-based mRNA copy number and a micro-assay for determining ACHE activity in samples containing single isolated NMJs. ACHE mRNA is an intermediate message at the NMJ compared to internal standard used (pACONT) and to co-actin mRNA levels measured in the same samples. ACHE message levels in non-innervated regions of the muscle fibers are either undetectable or very low, making the NMJ a rare transcriptional fiber region. Analysis of more than 50 individual NMJs shows that the ACHE transcript levels are highly variable (more than 20 fold range) yet were detected in only 36% of our sample NMJ samples. In contrast, analysis of ACHE activity in single NMJs showed that enzyme levels were remarkably constant (12.23 ± 1.4 pmol of ACh hydrolyzed/muM; mean ± S.D.). These results show that ACHE gene is compartmentalized at the NMJ and provide a mechanism for regulating the abundance of this synaptic protein at sites of nerve-muscle contact. Furthermore, the observed variability in ACHE transcript levels compared to enzyme activity suggests that transcription of this synaptic protein gene may occur intermittently rather than constitutively possibly reflecting a regulated transcriptional control mechanism linked to muscle activity. Supported by NIH and MDA grants to R.L.R.

649.2 ACETYLCHOLINESTERASE mRNA LEVELS INCREASE IN PARALLEL WITH ENZYME ACTIVITY IN OVERLOADED SKELETAL MUSCLE. B.J. Jasmin, R.K. Lee and R.L. Rotundo. Department of Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33101.

Normal expression of acetylcholinesterase (ACHE) in vertebrate skeletal muscle depends upon the presence of the nerve as well as normal contractile activity. In general, denervation of a muscle leads to regression of the ACHE gene in the collagen-tailed ACHE form whereas muscle overload induces significant increases in specific ACHE oligomeric forms. These observations indicate that expression of ACHE can be modulated according to the metabolic state and the load the muscle experiences. As a first step towards understanding the molecular mechanisms underlying the activity-induced ACHE plasticity in muscle we subjected adult quails to an overload model that results in a dramatic enhancement of muscle force. We have performed comparisons of enzyme activity, ACHE isoform distribution, and morphology of the overloaded muscle vs. control muscle. The increased enzyme activity and ACHE isoform distribution is paralleled by fiber type specific expression of the ACTPase, SDH, and GPD activities. ATPase activity remained unchanged compared to control. No variation was seen in the collagen-tailed form of enzyme activity whereas muscle overload induced significant increases in the fast type form of ACHE (G2 and G1 displaying 32%, 122%, 230% and 210% increases, respectively). These increases in specific ACHE oligomeric forms. These observations indicate that expression of ACHE can be modulated according to the metabolic state and the load the muscle experiences. Supported by NIH Grant NS16333 & NS16333.


The coordination of isoform expression of proteins responsible for both contraction (myosin heavy chain, MHC) and relaxation (sarcoplasmic reticulum calcium ATPase, SR-ATPase) were evaluated in the soleus of cats undergoing a slow transition (67%) to fast transition, induced by spinal transection at T12-L1. Three groups of adult cats were used: 1) control; 2) spinal transected (ST); and 3) spinal transected and weight-supported (ST&WS). Serial cross sections were processed for immunohistochemistry using monoclonal antibodies to MHC and SR-ATPase isoforms. Fibers with the following MHC compositions were detected: Type I, Ia and Ila, IIx and IIX. The large shifts in MHC and SR-ATPase isoforms were correlated with the pattern of units defined in the intact muscle. The relative number of fibers was reduced to 67% in ST and 76% in ST&WS. Relatively few fibers transected with daily weight support exercise (ST&WS). The increased enzyme activity and ACHE isoform distribution is paralleled by fiber type specific expression of the ACTPase, SDH, and GPD activities. ATPase activity remained unchanged compared to control. No variation was seen in the collagen-tailed form of enzyme activity whereas muscle overload induced significant increases in the fast type form of ACHE (G2 and G1 displaying 32%, 122%, 230% and 210% increases, respectively). These increases in specific ACHE oligomeric forms. These observations indicate that expression of ACHE can be modulated according to the metabolic state and the load the muscle experiences. Supported by NIH Grant NS16333 & NRSA (DE07212) from NIDR.


The effects of chronic electrical inactivity on the myosin heavy chain (MHC) composition and succinic dehydrogenase (SDH) activity were assessed in the anterior tibialis (TA) muscles of adult cats subjected to complete denervation. Animals were divided into three groups: 1) control; 2) spinal transected (ST); and 3) spinal transected and high intensity weight-supported exercise (ST&WS). Total myosin heavy chain (MHC) composition was assessed using SDS-gel electrophoresis to differentiate between type I and II fast (IIa) and slow (IIx) MHC, and type I and type II heavy chain isoforms (I and Ia). The coordination of isoform expression of proteins responsible for both contraction (myosin heavy chain, MHC) and relaxation (sarcoplasmic reticulum calcium ATPase, SR-ATPase) were evaluated in the soleus of cats undergoing a slow transition (67%) to fast transition, induced by spinal transection at T12-L1. Three groups of adult cats were used: 1) control; 2) spinal transected (ST); and 3) spinal transected and weight-supported (ST&WS). Serial cross sections were processed for immunohistochemistry using monoclonal antibodies to MHC and SR-ATPase isoforms. Fibers with the following MHC compositions were detected: Type I, Ia and Ila, IIx and IIX. The large shifts in MHC and SR-ATPase isoforms were correlated with the pattern of units defined in the intact muscle. The relative number of fibers was reduced to 67% in ST and 76% in ST&WS. Relatively few fibers transected with daily weight support exercise (ST&WS). The increased enzyme activity and ACHE isoform distribution is paralleled by fiber type specific expression of the ACTPase, SDH, and GPD activities. ATPase activity remained unchanged compared to control. No variation was seen in the collagen-tailed form of enzyme activity whereas muscle overload induced significant increases in the fast type form of ACHE (G2 and G1 displaying 32%, 122%, 230% and 210% increases, respectively). These increases in specific ACHE oligomeric forms. These observations indicate that expression of ACHE can be modulated according to the metabolic state and the load the muscle experiences. Supported by NIH Grant NS16333 & NRSA (DE07212) from NIDR.

649.5 EMG/FORCE RATIO AS AN INDICATOR OF MUSCLE CELL DEATH FOLLOWING ECCENTRIC EXERCISE. J.N. Howell*, G. Chleboun, D. Gardensky, M. Wend, and R.J. Roy*. Department of Physiological Science and Brain Research Institute, UCLA, CA, 90095.

Following eccentric exercise to failure of the human elbow flexors under heavy load (90% of isometric max.); elbow flexion (~ 90°), increased 2 to 3 fold (N=37). Associated with failure was a decrease in mean frequency necessary to maintain 50% and 70% of P˳ ˳ (J Histochem Cytochem 35:1037, 1987). Optical density measurements from glycyogen-stained frozen sections were used to classify fibers as depleted (unit) or non-depleted (non-unit). Myofibrillar ATPase activity was determined as described by Jiang et al. (Muscle & Nerve 13: 1037, 1990). SDH and GPD activities were determined as described by Martin et al. (J Histochem Cytochem 53:1055, 1995). MHC composition was assessed using monoclonal antibodies specific for slow and fast MHCs (Contracted by S.Schaffer, ParkRow, CA). Three groups of units were defined: 1) control; 2) spinal transected (ST); and 3) spinal transected and high intensity weight-supported exercise (ST&WS). The increased enzyme activity and ACHE isoform distribution is paralleled by fiber type specific expression of the ACTPase, SDH, and GPD activities. ATPase activity remained unchanged compared to control. No variation was seen in the collagen-tailed form of enzyme activity whereas muscle overload induced significant increases in the fast type form of ACHE (G2 and G1 displaying 32%, 122%, 230% and 210% increases, respectively). These increases in specific ACHE oligomeric forms. These observations indicate that expression of ACHE can be modulated according to the metabolic state and the load the muscle experiences. Supported by NIH Grant NS16333 & NRSA (DE07212) from NIDR.


The fatigue resistance of motor units in the soleus muscle has been appreciated for some time. Recently, however, it was shown that the relative fatigue resistance among these units can vary considerably. Using cats, we have assessed the coordination of units to fatigue resistance among motor units can undergo a form of potentiation which operates at the MU level following chronic inactivity. (Supported by NIH Grant NS16333).
649.7

MUSCLE I

1557
gene expression of contractile proteins. (Supported by Canadian MRC and
muscles which account for the wide range of MU CTs, despite the change in
MDAC and AHFMR).

rate limiting for CT and 2) that differences between fibers remain in stimulated
showed that, at 6 weeks, muscles were heterogeneous in composition but long-
no evidence of hybrid fibers. The finding that all MUs become slower without
However, the range of values was NOT greatly reduced and did not correspond
press). We analyzed up to 14% of the total MU population in the cat MG at 6
a gradual increase in twitch contraction time (CT) and fatigue resistance
electrical stimulation (FES; 20Hz, 50% duty cycle for at least 2 hrs/day), caused
homogeneous motor unit (MU) population occurs. In medial gastrocnemius
Alberta, Alta T6G 2S2 and Dept. Physiol., Univ. Ottawa, Ontario K1H 8M5,
Canada.

MOTOR UNIT HETEROGENEITY FOLLOWING FUNCTIONAL
STIMULATION OF CAT AND HUMAN MUSCLES. M.C. Patullo, V.E.
Refase, D.J. Parry and N. Tyerman. Dept. Pharmacology., Div. Neuroscience., Univ. of
Alberta, Alta T6G 2S2 and Dept. Physiol., Univ. Ottawa, Ontario K1H 8M5, Canada.

Although it is well recognized that whole muscle proteins can be modulated by
imposed activity, it is not known whether the predicted conversion to a
heterogeneous motor unit (MU) population occurs. In medial gastrocnemius
(MG) in the cat, the tibial anterior (TA) in spinally injured patients, functional
electrical stimulation (FES; 20Hz, 50% duty cycle for at least 2 hrs/day), caused
a gradual increase in twitch contraction time (CT) and fatigue resistance
We analyzed up to 14% of the total MU population in the cat MG at 6
(weeks) and the rest at 16-32 weeks after continuous FES (long-term FES), in both cat and human, increase in mean values of MU CT and
fatigue indices corresponded with changes in whole muscle properties.
However, the range of values was NOT greatly reduced and did not correspond
to the range of the MU populations examined. Immunohistochemical analysis
of myosin heavy chain (MHC) isoforms in stimulated cat muscles showed that,
at 6 weeks, muscles were heterogeneous in composition but long-
term stimulation resulted in a homogeneous muscle composition with no evidence of hybrid fibers. The finding that all MUs become slower without
reducing the range of CT in the MU population provides evidence that 1)
factors other than MHC content which include Ca release and uptake rates, are
rate limiting for CT and 2) that differences between fibers remain in stimulated
muscles which account for the wide range of MU CTs, despite the change in
gene expression of contractile proteins. (Supported by Canadian MRC and
MDAC and AHFMR).

649.8

DIFFERENCES IN FATIGABILITY OF MOTOR UNITS OF THE SAME
TYPE NOT RELATED TO DIFFERENCES IN ENERGY-METABOLISM. S.
Snodida, R.J. Callister, P.M. Nenoth, R.M. Entowa, R.M. Retnak and D.G.
Stuart. Dept. of Neurology, Wash. Univ. Med. Sch., St. Louis, MO 63110 and
Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724.

Earlier studies on muscle fatigue led to the recognition that resistance to fatigue
of motor-units of different histochemical types was positively related to the
activity of energy-generating enzymes of the oxidative pathways. In this study
we have examined the relationship between energy metabolism and fatigue in
motor-units of the same histochemical type but of differing fatigability. Both
single axons to rat EDL muscles were stimulated to fatigue-fast-fatigable (FF)
motor-unit muscle fibers. After the fatigue test, the motor unit was continuously
stimulated at 40 Hz for one hour to deplete its muscle fiber of ATP. The muscles
were then rapidly dissected, map frozen in liquid nitrogen and mounted on
blocks for cross-sectioning. Sections of thick (56 μm) and thin (14 μm) cross-
sections were cut from the muscle with the thick sections being frozen
then stored under vacuum at -70°C. Thin sections were stained for glycogen
Periodic acid-Schiff (PAS) reaction) and myosin adenosine triphosphatase.
Glycogen depleted fibers as identified from the PAS-stained sections were
dissected from the thin freeze-dried cross-sections. The fibers were divided into
categories, weighed on quartz-fiber balance and assayed for activity of
enzymes belonging to different metabolic pathways (i.e. glycolytic, high
energy phosphate). Our preliminary results show that while a difference in
fatigue index (and hence fatigability) exists, no correlation difference exists in
the activity of the enzymes of energy-generating and energy-consuming enzymes and fatigability. We are presently
examining this aspect in further detail.

649.9

IDENTIFICATION OF OPTIMAL INTERPULSE INTERVAL (IPI)
PATTERNS FOR ACTIVATION OF FATIGUED HUMAN QUADRICEPS
PERENNIS MUSCLE. S.A. Binder-Macleod and Scott Randels. School of Life
and Health Sciences, Univ. of Delaware, Newark, DE, 19716.

This study attempted to identify the stimulation pattern during brief (10-
second) bursts, submaximal trains of pulses that produced the greatest
force when the muscle was fatigued by repetitive activation. Each train lasted = 60
sec; rest periods of ~5-600 sec separated each train. Subjects (N=8) participated in three experimental sessions. During the first session, each subject was
stimulated with constant frequency trains (CFTs, all IPI durations ~ 70 ms) and
five variable frequency trains (VPTs, first IPI duration ~ 5, 10, 15, 20 or 30; the
remaining IPI duration ~ = 70 ms). During the second experimental session, the second IPI duration was varied while the first IPI was kept at the
interval that produced the greatest force during the first session. During the
third session, the second IPI duration was varied. The sequencing of trains within
each session was random, with each train repeated 30 times during each session
(180 contractions). By the 120th contraction a stable level of fatigue was noted.

The mean force produced during the last 10 contractions for each stimulation
pattern was calculated to compare the response of the muscle to each stimulation
pattern. The results showed that one short IPI at the onset of the train produced 32%
more force than the CFT, two short IPIs 64%, and three short IPIs 29%. Thus,
the results may have significant implications when electrical stimulation is used clinically
to activate skeletal muscle reproducibly. Such applications include stimulation of lower extremity muscles to assist spinal cord injured patients to ambulate and
stimulating of the latissimus dorsi muscle to assist cardiac muscle function.

649.10

INNERVATION RATIO IS THE MAJOR DETERMINANT FOR THE WIDE RANGE
OF MOTOR UNIT FORCE IN THE CAT MEDIAL GASTROCNEMIUS (MG)
of Neuroscience, Univ. of Alberta, Canada. T6G 2S2.

The extent to which motoneuron branching (innervation ratio; IR) determines the normal variation in motor unit (MU) force within a single muscle is a contentious issue. Determining the range of unit IRs in a large heterogeneous muscle such as the cat MG muscle has been difficult due to 1) the wide range in muscle fiber cross-sectional areas (CSAs) between different MU types and 2) counting all muscle fibers by glycogen depletion is difficult due to the steep pinnaeh of muscle fibers. These problems for calculating the IR of MUs in the cat MG muscle have been overcome in recent experiments with long-term low-frequency electrical stimulation. Cat MG muscles were selectively stimulated (20Hz, 50% duty cycle) for 6 to 32 sec via a MG nerve cuft electrode attached to a small portable stimulator fastened to the cat’s back. In a preliminary experiment the number of fibers was determined by isolating and characterizing at least 14% of the MG MUs. The muscle fibers of a single MU were also depleted of glycogen by repetitive stimulation at a low
frequency. The MU muscle was then dissected for histological and histochemical analysis. Following 19 ± 10 seconds of stimulation the normal 100- fold range in unit force was reduced to a 40- fold range. Concurrently, the range of CSAs for the whole muscle fiber population was similarly reduced from an 8 to 4- fold range. Normally, the range in muscle fiber CSAs within a single MU is 50% of that of the whole muscle fiber population. Following stimulation the mean and range of muscle fiber CSAs within a single MU was the same as for the entire muscle. Since the mean muscle fiber CSA of all MUs became similar and IR is not changed by stimulation, these results provide strong evidence that the 40- fold range is IR of MG motoneurons. (Supported by MRC, AHFMR and NCE).

649.11

IMMUNOHISTOCHEMICAL ANALYSIS OF FIBER TYPES WITHIN
PHYSIOLOGICALLY TYPED MOTOR UNITS OF RAT TIBIALIS ANTERIOR
MUSCLE AFTER LONG-TERM CROSS-INNervation. M.C. Patullo, V.E.
Refase, D.J. Parry and N. Tyerman. Dept. Pharmacology., Div. Neuroscience., Univ. of
Alberta, Alta T6G 2S2, & Dept. Physiology. Univ. of Ottawa, Ont. K1H 8M5,
Canada.

We have previously shown in the rat (Totosy de Zepetnek et al., Soc.
Pharmacol. 68: 596-602, 1990) that the characteristic distribution of MUs in the
depth and superficial regions of Tibialis Anterior (TA) is also seen after self-innervation, under conditions in which nerves do not reinervate their former muscle fibers. In this study, we have used antibodies to type I, Ila and Iib myosin heavy chains (MHCs) to investigate 1) the spatial distribution of fiber types 1 year after cross-innervation of rat TA by posterior tibial nerve and 2) the fiber type composition of characterized MUs. We found that the spatial distribution of fiber types was not statistically different from normal despite increased clumping of fiber types. Fibers which did not react with any of the 3 antibodies, presumably type IIx fibers, comprised up to 30% of the total, similar to the proportion of FI MUs in normal and self-innervated muscles (Totosy de Zepetnek et al., J. Neurophysiol., 67 #5, 1992). Within identified fast MUs, most fibers depict muscle fiber composition in which was congruent with the MU type (i.e. FF=IIB, FR=IIA). However, there was considerable variation in the intensity of staining and a significant proportion of fibers (>5%) in FF and FR MUs appeared to be type IX. In FI MUs, 5-10% of fibers were
fibral positive. These findings provide suggestive evidence for an intrinsic
regulation of muscle fiber type which allows for the maintenance of the original spatial distribution and may account for the fiber heterogeneity within a single
MU. (Supported by MDAC, MRC and AHFMR).

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649.13

NEUROLOGICAL ALTERATIONS OF GLYCOCEN PHOSPHORYLASE EXPRESSION IN RAT SKELETAL MUSCLE C. C. Matthews and R. C. Carlsen* Department of Human Physiology, School of Medicine and Dentistry, University of Rochester, Rochester, NY 14620.

Denerveated skeletal muscle undergoes a series of metabolic changes, including a decrease in both the activity of glycogen phosphorylase and the content of muscle-specific glycogen phosphorylase messenger RNA. We tested the hypothesis that the loss of neurotrophic substances following denervation is responsible for the decrease in MGP transcription. Several concentrations of vinblastine were used to block axonal transport in situ in the rat peroneal nerve. Nerve conduction velocities and tibialis anterior contractile properties were measured at 7 days after initiation of the axonal transport block. MGP expression in the TA muscle was determined using standard Northern analysis procedures. The results show that axonal transport of acetylcholine increases decreased activity declined in nerves treated with 0.2% w/v or 0.4% vinblastine. There was also a dose-dependent decline in motor and sensory nerve conduction velocities one week after treatment with 0.2% or 0.4% vinblastine. The ability of the muscle to produce force likewise declined with the highest doses of vinblastine. The amount of MGP mRNA present 7 days after vinblastine treatment decreased substantially when the nerve was exposed to 0.4% vinblastine. A lesser decrease in MGP mRNA developed after treatment with 0.2% vinblastine. Lower concentrations of vinblastine produced no apparent change in the content of MGP mRNA. These results suggest that MGP transcription is under neurotrophic regulation, but by some means other than the release of an axonally transported diffusable substance.

650.1


The pectoralis muscle of the pigeon (Columba livia) is composed of fast oxidative glycolytic (FOG) and fast glycolytic (FG) fibers thought to be differentially recruited during take-off and steady flight. To assess the neuromuscular organization of FOG and FG motor units, pectoral muscle unit architecture and motor pool organization were studied with glycogen depletion and retrograde axonal tracing techniques. Individual motor units were identified by the loss of one or two fiber populations. Three FOG units were 2.4, 2.5 and 3.5 cm in length, and consisted of 100, 250 and 300 fibers respectively. A single FG unit was 3.5 cm in length and consisted of 170 fibers. None of the muscle units extended more than 30% of the origin-to-insertion whole muscle length. Following application of a 30-40% solution of HRP to cut pectoral nerves (N=3), retrogradely labeled neurons were located in cervical segments 10 to 12. Motoneuron soma area ranged from 3000-18000 sq µm (% 9000 sq µm). A few exceptionally large neurons (15000-18000 sq µm) were observed in each bird. These results 1) demonstrate that individual muscle units occupy a small portion of the pigeon pectoralors muscle and do not extend from muscle origin to muscle insertion and 2) suggest that force transmission in the pectoralors proceeds through myo-vascular and/or endomysial connections between the fibers of in-series muscle units. The presence of large motoneurons in the pectoralors motor pool suggests a possible correspondence between motoneuron size and muscle unit area and/or fiber type. Supported by NSFC grant DCB-8718727.

650.3

FIBER TYPE DIVERSITY IN THE VIBRISsal FACIAL MUSCLES OF RODENTS. L. E. Winick*, S. A. Pitts and D. J. Weeks. Dept. Anatomy, Morehouse School of Medicine, Atlanta, GA 30310; Dept. Biological Sciences, Florida International University, Miami, FL 33199.

The golden hamster (Mesocricetus auratus), Norway rat (Rattus norvegicus), and guinea pig (Cavia porcellus) exhibit three types of vibrissae is very similar in the three species; however, the muscles may differentially recruited during take-off and steady flight. To assess the exploratory behavior, as defined by the use of the mystacial vibrissae. We compared the populations of intermediate-level fibers. In general, the vibrissal muscles are composed almost entirely of fast-twitch, fatigue-resistant fibers with minor populations of slow-twitch, fatigue-resistant fibers. However, there is great diversity in the subtype composition of the muscles. Supported by NIH 506-GM8248 and NIDRR.

650.4


The succinate dehydrogenase activity (SDH) of muscle fibers from the sexually dimorphic levator (LA) and bulbocavernosus (BC) muscles were determined using a quantitative histochemical technique from cryostat sections of fresh frozen tissues. The betaine and male SDH activities were equal in both the BC and LA muscles. Furthermore, the BC and LA muscle fiber populations were metabolically homogenous. For type IIB fibers the mean SDH activity was greatest among fibers from the deep region of the MG followed in descending order by the superficial region of the MG, the BC, and the LA muscles. These results suggest that the BC and LA muscles are composed of highly fatigable fast-twitch muscle units. (Supported by grants HL 34017 and HL 37860.)
560.5  

We have shown previously in mammals (adult cat) that sudden changes in motor unit firing patterns can delay and reduce fatigue (Bevan et al., J. Physiol., London 1996). To extend on these observations, we have recently changed our animal model from cat to turtle (P. scripta) as this species affords a unique opportunity, in vertebrates, to study segmental motor mechanisms in intact (in vivo), in vitro (slice) and culture-preparations. In this report, we examine the catch-like property in the whole external gastrocnemius muscle (EG; 27% SO, 34% FOG and 44% Fg fibers) during shortening and lengthening contractions, and following fatiguing isometric contractions. Two different stimulation regimes, constant-frequency (10 pulses, 100 ms intervals) and catch-inducing (two additional 10 ms intervals inserted at the beginning of the constant-frequency pattern), were applied to the EG muscle nerve. The force-time integral attributable to each stimulus pattern was quantified. The catch-inducing pattern always produced greater force than the constant-frequency pattern. However, the magnitude of the increase depended on the type of contraction. A comparison of force enhancement, due to the catch-like property, prior to and during shortening (17% vs. 14%) and lengthening (20% vs. 16%) contractions did not reveal any significant differences. This contrasts with the marked difference (12% pre-fatigue vs. 37% post-fatigue) in force enhancement observed in muscles following a period of fatiguing (10 Hz trains, 10 p/min, 1 train/2 s for 4 mins) isometric contractions. It remains to be determined if a certain becomes more important during dynamic contractions in fatiguing muscle, as is the case for isometric contractions. Supported by USPHS grants GM 08400, HL 07244, NS 25077, NS 07309, NS 20544, and NS20762.

560.6  
SARCROME LENGTH-JOINT ANGLE RELATIONSHIPS OF SEVEN FROG HINDLIMB MUSCLES. B.L. Lieber* and C.G. Brown. Department of Orthopaedics and Biomedical Sciences, Graduate Group, U.C. San Diego, School of Medicine, La Jolla, CA 92037-1099.

The relationship between muscle length and joint angle for a given muscular system often appears to be unique. In order to determine whether this is true, we have analyzed the length-angle relationship of seven muscles of the hindlimb (Rana pipiens). Muscles studied included the cruralis, iliacus internus, gastriomeniscus, semitendinosus, biceps femoris, cruralis, and semimembranosus, and the semitendinosus. Muscle-joint complexes were mounted in a jig and submersed in chilled Ringer's solution. Joints were rotated throughout their range of motion while sarcorme length was measured by laser diffraction. Sarcrome length change per degree of joint rotation (i.e., dL/dθ) ranged from a low of 3.7 mm/° for the cruralis muscle acting at the knee to a high of 12.5 mm/° for the semitendinosus muscle acting at the hip. dL/dθ values for muscles acting at the hip joint were significantly greater than those for muscles acting at the knee (p<0.005). dL/dθ was also negatively correlated with fiber length, suggesting a balance between fiber length and moment arm in most muscle-joint systems. Many exceptions to this generalization were noted. These data suggest that various muscle-joint combinations are "designed" for differential contribution of muscle force production to the joint torque profile.

560.7  
ELECTROPHYSIOLOGICAL PROPERTIES AND CALCIUM ACTIVATED POTASSIUM CHANNELS IN RAT CEREBROVASCULAR SMOOTH MUSCLE CELLS. Y. Wang and D.A. Malherbe. Department of Physiology, University of British Columbia, Vancouver B.C., Canada V6T 1W5.

Patch clamp methods were used to study the electrophysiological properties and the calcium activated potassium channels of smooth muscle cells (SM C s) from the cerebral arteries of adult Wistar rats. Procedures were developed for the enzymatic dissociation of these cells. Dissociated cells were maintained at 4°C for 1-3 days prior to use. Whole cell and inside-out patch clamp recordings were made at 21°C using a List EPC-5 amplifier. During whole cell current clamp recordings from these cells, the resting membrane potential was found to be -41 mV. Application of strong depolarizing stimuli evoked only small responsive generators and full action potentials were not observed. When measured at zero applied current, the slope resistance was 5.2 GΩ. The average membrane time constant was 78 ms and cell capacitance was 24 pF.

Isolated, single-barreled patches excised from these cells displayed calcium activated potassium channels with intermediate single channel conductance (92 pS) in symmetrical 140 mM KCI solution. These channels were activated by both increasing intracellular free calcium and depolarization of the patch membrane. Tetradotoxin caused a reversible, dose-dependent reduction in the amplitude of current in the channels, when applied to the cytoplasmic membrane face (IC50=0.31 mM).

560.8  
CYTOARCHITECTURE OF THE NEUROMUSCULAR JUNCTION IN DIABETIC MUSCLE. K. M. Klueber* and S. Stansel. Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, KY 40292.

During the pathogenesis of diabetes, a peripheral neuropathy occurs in the C57Bl/6J-dbm diabetic mouse. Prior work indicated that neuromuscular remodelling occurs in this mouse (Klueber and Stahl, Neurosci. Abstr. 16:151, 1988). The objective of the present study was to evaluate the cytoarchitecture of these neuromuscular junctions (NMJ) to provide an index of remodelling. The extensor digitorum longus muscles from young (8wk) and old (20wk) female diabetic and control mice were examined electron microscopically. In the diabetic muscles, thirty percent of the NMJs examined at both time points exhibited various degrees of degeneration. Remodelling was also observed as indicated by the presence of sprouts (4%). Reinervation was indicated by NMJs exhibiting secondary synaptic clefts which extended beyond the axon (10%; 8wk; 5%; 20wk) while others exhibited only limited secondary cleft formation (13%; 8wk; 9% 20wk). Both of these profiles are characteristic of reinervation following nerve section and are hypothesized to be stages in the maturation of regenerating NMJs (Hansen-Smith, Anat. Rec. 207:55, 1983). Thus regeneration and remodelling of NMJs is more frequent in young than rather old diabetic muscle and occurs to a greater degree in diabetic than in normal muscle. (Supported by DK41853.)

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—CONDITIONING I

651.1  
DISRUPTING CEREBELLAR DEVELOPMENT IMPAIRS EYEBLINK CONDITIONING IN THE INFANT RAT. J.H. Freeman, Jr. and D.J. Freeman. *Department of Psychology, U.C. Irvine, Irvine, CA 92717; †Psychology Dept., UNC, Chapel Hill, NC 27514.

There is a dramatic increase in rate of eyeblink conditioning (EBC) between Postnatal Day 17 (PND17) and PND24 in the rat (Stanton, Freeman, & Shelton, Behav. Neurosci., 1988), which may reflect an important developmental stage of the cerebellum. To examine this possibility, we exposed pups neonatally to the anesthetic agent, m-Chloroform (nCM), a treatment that is known to impair the development of cerebellar cortex, while leaving most other brain structures intact (Chen & Hillman, 1986, Brain Res., 352:431). On PND20 and PND21, rat pups were injected (s.c.) with saline or 20 mg/kg nCM. On PND21 or PND22, they were trained on spatial delayed alternation, a task that is sensitive to early limbic and prefrontal cortical damage (Freeman & Stanton, in press; 1991, Behav. Neurosci., 105, 386-395). On PND23 or 24, they were trained on EBC (see Stanton et al., in press). Pups exposed to nCM were not impaired on delayed alternation (suggesting no functional impairment of hippocampus or prefrontal cortical). In contrast, pups that showed nCM-induced cerebellar hypoplasia were impaired on acquisition of EBC. These findings suggest a role for cerebellar development in the ontogeny of EBC.

651.2  

Initial studies have demonstrated that associative eyeblink conditioning (EBC) can be established in the infant rat (Stanton, Freeman, & Shelton, Behav. Neurosci., in press). One purpose of this research is to develop a preparation for the study of the developmental stages of the ontogeny of learning. As a first step toward this end, we sought to determine whether the cerebellum was critical for EBC acquisition in the infant rat. In the adult rat, lesions of the cerebellar deep nuclei abolish EBC (Shelton, Behav. Neurosci., 1988). However, it is not known whether early cerebellar damage would affect acquisition of EBC in the infant rat.

Pups received either sham surgery or cerebellar removal by aspiration (portional Day 10). On PND22, all pups were trained on EBC (see Stanton et al., in press). Animals with complete cerebellar removal were dramatically impaired when compared with animals in the sham-surgery group. This experiment extends and confirms the structure-function homologies between the cerebellum in the rat and rabbit. Moreover, the results suggest a role for the cerebellum in the ontogeny of EBC.
651.3 EVIDENCE THAT THE PRINCIPAL ABDCENS NUCLEUS UNDERLIES THE IN VITRO CONDITIONED EYE-BLINK RESPONSE. J. Keifer*, Dept. of Physiology, Northwestern Univer. Medical School, 303 E. Chicago Ave., Chicago, IL 60611.

Two distinct populations of abducens motor neurons contribute to the eye-blink reflex: the principal and the accessory abducens nuclei. Accessory abducens sends its axons to the retrobulbar mass of muscle and receives afferents from the trigeminal nucleus and premotor blink areas. Principal abducens projects to the lateral rectus and retractor bulbi muscles. The population input from the trigeminal nucleus is smaller than that of the accessory abducens nucleus and half of the population axons end in the red nucleus during conditioning. The proportions of the principal and accessory abducens nuclei are different depending on the relative contribution of the abducens nucleus to the conditioned response (CR). Previous studies have found that the in vitro turtle brainstem-cerebellum was a useful model to study the conditioned eye blink reflex. Using the activity-dependent dye sulfofluorochrome, evidence has been obtained suggesting that the principal abducens nucleus, rather than the accessory abducens nucleus, has a predominant role in producing the conditioned response. Paralyzed electrical stimuli to the posterior nerve VIII (CS) and the trigeminal nerve (UCS) were applied to the in vitre preparation while recording activity in the abducens nerve as described previously (Soc. Neurosci. Abs. 16: 763, 1990). Once a conditioned response had been acquired, sulfofluorochrome was added to the bath and the preparation was given CS-only stimuli. Hence, during dye application, only CR's were recorded. The results show that during expression of the CS, labeled neurons were found predominantly in the principal abducens nucleus as compared to the accessory abducens nucleus. Labeled neurons were also observed in the red nucleus and lateral cerebellar nucleus, areas which never label during the UCR. Purkinje cells, the reticular formation, and the cochlear nucleus also labeled with dye.

The results suggest that the principal abducens nucleus is in the modifying pathway leading to the CR. It is intriguing since activity in this nucleus, but not in the accessory abducens, is modulated by NMDA. Although not traditionally considered, the principal abducens and accessory abducens nuclei, both of which contain CS-US convergence, are postulated to be potential sites of learning. (NSF BNS-9109572)

651.4 PERFORMANCE OF UNCONDITIONED NICTITATING MEMBRANE RESPONSES IN THE INTACT RABBIT IS AFFECTED BY MUSCIMOL INACTIVATION OF THE CEREBELLUM. V. Braida*, M.J. Webster, J.R. Bledsoe, Barrow Neurological Institute, Phoenix, AZ 85040.

The purpose of this study was to examine the specificity of involvement of the anterior interpositus nucleus (AIN) in the control of nictitating membrane reflexes. Animals were trained in the timed delay paradigm using 450 ms sound as the conditioned stimulus and 100 ms unilateral corneal air-puff as the unconditioned stimulus (ISI=350 ms, ITI=17-23 s). The trained animals were injected with muscimol in the AIN ipsilateral to the trained eye and tested in experiments in which trials consisting of paired conditioning produced different subsets of unconditioned stimuli were alternated with trials containing the unconditioned stimulus alone.

The muscimol microinjections (200 ng) completely abolished the conditioned responding. Explicit testing of the unconditioned reflex before and after drug administration revealed that activation of GABA-A receptors significantly decreases the amplitude of the unconditioned nictitating membrane responses. The analysis of behavior in animals which developed bilateral conditioned responses indicate that the drug effect is strictly restricted to the ipsilateral eye.

The results of the present study do not support the notion that the AIN is involved exclusively in mediating the classically conditioned nictitating membrane reflex. The behavioral effects of muscimol injections in the AIN suggest that this cerebellar nucleus participates in control of both conditioned and unconditioned nictitating membrane responses in intact rabbits. NIH Grant R01 NS21585.


New Zealand White rabbits were implanted with cannulae in the dorsal or ventral aspect of the anterior interpositus nucleus. Three days (and six days for some animals) of standard tone-airpuff training was given with continuous infusion (constant rate of 0.2 nl/min) of Lidocaine (2, 4, 8, 16%) or saline. All animals were then given three days of training with no infusion. The minimum dose (concentration) of Lidocaine necessary to abolish performance of the CR was then determined for every animal. Saline control animals learned to criterion during the three days of infusion training. Lidocaine animals with dorsal cannula locations and appropriate doses exhibited no CRs in the three (or six) days of infusion training and learned in the subsequent three days of no-infusion training as if naïve, i.e. they exhibited no savings. Animals with ventral cannula locations and appropriate doses showed no CRs during Lidocaine infusion training but substantial savings in subsequent no-infusion training. Thus both acquisition and performance of the CR were completely abolished, depending on cannula location, in a dose dependent manner. These results strongly suggest that the principal abducens nucleus, an appropriate memory trace for eyeblink conditioning is formed and stored in the cerebellum. (Supported by NSF and ONR grants to R.F.T.)


The GABA agonist muscimol was used to assess the cerebellum's role in eyeblink conditioning. In well trained rabbits, varying concentrations of muscimol were infused into the cerebellum through chronically implanted cannulae aimed at the ipsilateral interpositus nucleus. Conditioned responses (CRs) were completely blocked (up to 8 hours with no effect on unconditioned responses (URs)). By 12 hours, CRs returned to pre infusion levels.

A group of naive rabbits (n=6) received 6 days of tone-airpuff conditioning. On the first 3 days, muscimol was infused into the cerebellum prior to training. Infusions of muscimol following training and subsequent histology confirmed that the muscimol was localized to the dentate/interpositus nuclei and overlying cortical regions. Another group of rabbits (n=10) received infusions of muscimol into the red nucleus on days 1-3. These rabbits showed no CRs on those days but showed a significantly higher percent CRs on day 4 than the cerebellum group.

These results demonstrate that the cerebellum is required for acquisition and expression of eyeblink conditioning. The results also indicate that the cerebellum is a focus for storage of this memory. Supported by NSF, ONR, and McKnight grants to RFT.

651.7 REVERSIBLE LESIONS OF THE RED NUCLEUS DURING ACQUISITION AND RETENTION OF A CLASSICALLY CONDITIONED BEHAVIOR IN RABBITS. Robert E. Clark* and David G. Lavoie, Neuroscience Program, University of Southern California, Los Angeles, CA 90089-2520.

We have previously shown that temporary cooling of the interpositus nucleus prevents acquisition of a classically conditioned eyeblink. In the present study we assess the role of the red nucleus during conditioning. A cooling probe was implanted lateral to the red nucleus. Recording electrodes were implanted in the right red nucleus and the left interpositus nucleus. Animals were trained for five days with the cooling probe activated. No behavioral conditioned responses (CR) developed. Controls were responding at a high percentage by day 3. Average UR amplitude on day 3 of normal training (cooling probe inactive) to a single pulse CS, rabbits learn differently timed responses at criterion levels. We hypothesize that temporal discrimination occurs via the activation of different mossy fibers. While the ISI range that supports conditioning is much smaller with a single pulse CS, rabbits learn differently timed responses at criterion levels. We hypothesize that temporal discrimination occurs via the activation of different mossy fibers. Peak amplitude for all CRs occurs approximately when a constant set of mossy fibers in the MCP are activated, along with the lesion data, suggests that a temporal discrimination mechanism exists in the cerebellar cortex. To distinguish these possibilities we tested the ability of individual animals to acquire differently timed CRs using stimulation of mossy fibers in the cerebellum. To test this hypothesis further, we trained rabbits with single pulse stimulation as the CS to produce temporally mismatched different subsets of unconditioned responses to criterion levels before degeneration of the electrode. Peak amplitude for all CRs occurs approximately when the US is presented. The animals were given differently timed pulses to the US when a constant set of mossy fibers in the MCP are activated, along with the lesion data, suggesting that a temporal discrimination mechanism exists in the cerebellar cortex. To test this hypothesis further we trained rabbits with single pulse stimulation as the CS to produce temporally mismatched different subsets of unconditioned responses to criterion levels before degeneration of the electrode. Peak amplitude for all CRs occurs approximately when a constant set of mossy fibers in the MCP are activated, along with the lesion data, suggesting that a temporal discrimination mechanism exists in the cerebellar cortex. To test this hypothesis further we trained rabbits with single pulse stimulation as the CS to produce temporally mismatched different subsets of unconditioned responses to criterion levels before degeneration of the electrode. Peak amplitude for all CRs occurs approximately when a constant set of mossy fibers in the MCP are activated, along with the lesion data, suggesting that a temporal discrimination mechanism exists in the cerebellar cortex. Supported by the National Institutes of Health and the National Science Foundation.
651.9

EFFECTS OF REWARDING ELECTRICAL STIMULATION OF LATERAL HIPOTHALAMUS ON CONDITIONING OF NICTITATING MEMBRANE RESPONSE IN RABBITS. L. Artiköö*, T. Korhonen, M. Penttönen, and T. Ruusuvirta. Dept. of Psychol., Univ. of Jyväskyla, P.O. Box 35, SF-40351, Finland

Possible facilitation or retardation effect of rewarding brain stimulation of lateral hypothalamic (ESB) was studied in classical conditioning of nictitating membrane responses in rabbits. A 250 ms train of ESB pulses were applied 250 ms after the unconditioned stimulus (UCS, corneal airpuff, 150 ms, 2.1 Ncm²). The conditioned stimulus (CS, tone 1000 Hz, 400 ms) preceded the UCS 250 ms in the control group and the CS-UCS pair by 250 ms. A tone CS was classically conditioned to an air-puff UCS followed by stimulation of the lateral hypothalamus. The rabbits showed increased orienting and activity to brain stimulation. Preliminary observations showed also that the air-puff UCS can act as a CS eliciting conditioned responses.

651.11

CINGULATE CORtical AND LIMbic THALamic NEURONAL RESPONSES TO UNEXPECTED CUE AND CONTEXTUAL STIMULUS DURING EXTINCTION OF DISCRIMINATIVE AVoidANCE BEHAVIOR IN RABBITS. A. Porombca*, Y. Kubota, E. Kang and M. Gabriel. Dept. of Psychol. and Beckman Institute, Univ of Illinois, Urbana IL 61801.

Hippocampal formation lesions enhanced the trained-inhibitory neuronal activity (TIA) in the anterior ventral (AV) thalamic nucleus during discriminative avoidance, training, whereas rabbits performed a locomotor conditioned response (CR) in an activity wheel to avoid a footshock signaled by a tone (CS+). After the shock, and they learned to ignore a tone (CS-) of different auditory frequency than the CS+, which did not predict shock (Gabriel et al., Exp Br Res. 1987, 67, 131-152). Unexpected training experiences such as extinction (CSs presented without shock to a trained subject) did not effectively suppress CR performance in rabbits with lesions, suggesting the hypothesis that CR suppression by unexpected events is due to TIA suppression in AV and possibly other limbic thalamic nuclei. Here, CRs were suppressed but anterior and medial dorsal (MD) thalamic and cingulate cortical TIA was not suppressed during initial trials of extinction with an unexpected context (altered background odor and illumination) or tone. These results disconfirm the hypothesis. TIA in anterior cingulate cortical area 24b, and in the anterior dorsal, magnocellular AV and MD thalamic nucleus was enhanced by the unexpected context (but not by the unexpected tone) during the first 20 extinction trials, relative to TIA during extinction trials without novelty. The novel context-induced AV thalamic TIA enhancement corroborated preliminary results of a study (Kang et al., Soc Neurosci Abstr. 1990, 16, 264) which showed loss of the enhancement in rabbits with lesions of Ammon’s horn. These results suggest that hippocampal eff erents mediate the enhanced TIA. The cortical and thalamic sites exhibiting the context-related enhancement of TIA may be part of a circuit involved in the immediate suppression of CRs and/or mnemonic encoding of novel information, but this circuit does not appear to be engaged by unexpected CSs. (Supported by NIH).
LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—CONDITIONING I

651.15 
UNCONDITIONED STIMULUS (US) EFFECTS DURING AN INTRACEREBELLAR STIMULATION PARADIGM. R.A. Swain* & R.F. Thompson Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Intracerebellar stimulation of leolbe HV1 white matter as US produces robust classical conditioning when paired with either tone or intracerebellar stimulation CS. CS alone presentations produce extinction and reinstatement of paired trials produces rapid reacquisition. In addition, explicit but not randomly unpaired presentations of the conditioning stimulus profoundly retard learning in subsequent acquisition trials (Swain, et al., 1992).

In the current experiment, we examined the nature of US elicitation of movement and any nonassociative effects of US preexposure on learning. Previous lesion results have underscored the essential role of the interpositus (IP) in the generation of movement in this paradigm (Swain et al., 1992). However, as HV1 stimulation activates Purkinje cell, mossy fiber and climbing fiber afferents to IP, we sought to determine the relative contribution of these fibers to the IP's generation of movement. 108 US trials of varying stimulus durations (50-300 msec) were presented to 7 rabbits. Analyses indicated that IP is differentially sensitive to climbing and/or mossy fiber input. US onset occurs within 100 ms of US onset eliminating the possibility that movement arises from rebound excitation of IP due to Purkinje cell hyperpolarization. Maximum UR amplitude, however, always occurs 50-100 msec from US offset.

The nonassociative effect of US preexposure on learning was examined in these same rabbits by presenting up to 10 days of paired CS-US trials. Comparison with nonpreexposed controls (N=9) indicated that US alone trials impair learning as measured by total number of trials to criterion and also by variability of CR performance from session to session.

Supported by NSF BNS-8718300, ONR N0001488K0112, & McKnight to R.F. Thompson.

651.16 

In earlier experiments we showed that electrical stimulation of cerebellar white matter elicits discrete motor responses of the facial and neck musculature; pairing a tone CS with the intracerebellar electrical US leads to the development of robust conditioned responses. The present experiment was designed to test for associative learning in a preparation in which both CS and US are delivered directly to the cerebellum. Chronic stimulating electrodes were implanted in rabbit cerebellum. Electrical US activating cortical parallel fibers and thence Purkinje cells, and an electrical US activating underlying white matter and eliciting unconditioned responses. Paired CS-US presentations led reliably to the development of conditioned responses; also, increased excitability was observed in cerebellar cortex. Pseudorandom unpresented pairings of CS and US did not produce any CRs, indicating that true associative learning, and not sensitization, is responsible for the observed effects. Subsequent pilot data suggest that similar conditioning may occur when intracerebellar electrical CS and US are delivered via the same intracortical electrode, at different intensities. This preparation provides a model for the study of plastic neuronal interactions within cerebellar networks critically involved in associative learning. (Supported by NSF BNS-8718300, ONR N00014-88K-0112, and the McKnight Foundation, to R.F. Thompson, and by the BDRC, University of North Carolina.)

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—CONDITIONING II

652.1 
SERAUM DIMORPHIC EFFECTS OF DEAMETHASONE ON ACTIVATION, LEARNING, AND CENTRAL MECHANIC ACETYLCHOLINE RECEPTORS IN RABBIT. M. H. K젼

Glucocorticoids have prominent roles in the modulation of behavior and the proposed psychoneuroendocrine interactions are sex dependent. The present study was undertaken to study the effects of deamethasone (DEX) on active avoidance learning and 3H-QNB binding in 6 brain regions. Male and female Sprague-Dawley rats (4 months) were injected with either 1 mg DEX in sesame oil or only the vehicle at 8:00 and were given active avoidance learning trials at 10:00. Following 5 days of learning trials, the rats were decapitated, brains removed and dissected. Blood corticosterone levels were measured. Receptor binding was determined in the cerebellum(c), hypothalamus(hyp), corpus striatum(stm), hippocampus(hip) and frontal frontal(frm), parietal(par), temporal(temp) and occipital(occ) cortices. ANOVA revealed significant differences between the groups with regard to corticosterone levels(p<0.01), learning performance at day 5 (p<0.05) and 3H-QNB binding in the c, hyp and par cortices (p<0.05). There was a significant correlation between learning performance at day 5 and corticosterone levels (p<0.001), 3H-QNB binding in the c, c, temp and par cortices (p<0.05). Correlations were also observed between corticosterone levels and receptor binding in the c, c, temp and occ cortices (p<0.05). Our results suggest that DEX effects active avoidance learning in rats through the mesocortical cholinergic system and more prominently in males.

652.2 
INVOVLEMNT OF THE LATERAL AMYGDALA AND PERIRHINAL CORTEX IN FEAR POTENTIATED STARTLE TO ACOUSTIC AND VISUAL STIMULI. S. Canepa* and M. Davis Dept. of Psychology and Psychiatry, Yale University School of Medicine, New Haven, Ct 06508.

Prior studies in our laboratory have indicated that post-training lesions of the lateral/basolateral complex of the amygdala or perirhinal cortex block fear-potentiated startle using a visual conditioned stimulus (CS). The goal of the present studies was to test the generality of the involvement of these nuclei in fear potentiated startle using an acoustic CS. Anatomical data show that the medial geniculate nucleus of the thalamic, the source of all auditory information to the forebrain, is strongly interconnected with the lateral nucleus of the amygdala and, cortically to the auditory and perirhinal cortex. The auditory cortex projects to the lateral nucleus of the amygdala, via a relay in the perirhinal cortex.

Rats received 10 pairings of a 70-db, 3.7-sec noise presented at 2 kHz, mixed with 10 pairings of a 3.7-sec fluorescent light, each terminating with a 0.5- or 0.6-mA footshock, presented as a variable intertrial interval of 2.5 min, on each of 2 consecutive days. To preoperatively match rats into groups with similar fear-potentiated startle, rats were assigned to groups based on the magnitude of fear-potentiated startle using a visual conditioned stimulus (CS). These groups were the following: Group 1 (n=16), control group; Group 2 (n=16), sham lesion group; Group 3 (n=16), lateral amygdala lesion group; Group 4 (n=16), perirhinal cortex lesion group; Group 5 (n=16), combined lesion group. To assess fear-potentiated startle, animals were placed in a 1.5 x 1.5 x 0.6-meter box with one wall made of lexiglass. A 0.5- or 0.6-mA electric shock was administered to the footpad. A noise CS was presented 0.5 sec before the footshock. The nonassociative impact of US preexposure on learning was examined in these same rabbits by presenting up to 10 days of paired CS-US trials. Comparison with nonpreexposed controls (N=9) indicated that US alone trials impair learning as measured by total number of trials to criterion and also by variability of CR performance from session to session. These results support the view that the inferior olive provides the unconditioned stimulus information to the cerebellum for classical eyeblink conditioning.

651.17 

Rabbits were classically conditioned using the delay paradigm with a tone conditioned stimulus (CS; 350 ms, 1KHz, 85 db) and an airpuff unconditioned stimulus (US; 100 ms, 3 psi, coterminating with the CS). Seven rabbits received bilateral motor cortex lesions prior to receiving 5 days of acquisition training. Another 7 rabbits received 5 days of acquisition followed by the lesion and 5 days of retention training. Each training session consisted of 12 blocks of 9 trials (1 CS-alone followed by 8 paired CS-US trials). Bilateral motor cortex lesions did not affect the acquisition or retention of the classically conditioned nictitating membrane response. The percentage of conditioned responses did not differ between lesioned and unlesioned animals during acquisition. Animals lesioned following acquisition showed no conditioned or unconditioned response deficits during subsequent training on any measure (percentage, amplitude, onset or peak latency, and amplitude-time area). Reflexive eyeblinks to 4 different US intensity levels (1, 2, 3, & 4 psi) measured over the course of training were unaffected by motor cortex lesions.

(Supported by NSF BNS-8718300, ONR N0001488K0112, & McKnight to R.F. Thompson.)

651.18 

This experiment investigated the effect of a GABA antagonist picROTOXIN (PTX) on the phenomenon of "blocking" using the conditioned eyelink response in the rabbit. Kamin's two-stage paradigm was employed. The blocking group received 7 days of tone-airpuff conditioning followed by 5 days of tone-light-airpuff compound conditioning. Half of the blocking animals received intra-olivary infusions of PTX (1 nM) during the compound conditioning phase, while the other half received artificial cerebrospinal fluid (CSF). The control group received 5 days of tone-light-airpuff compound conditioning only. Preliminary results indicate that the CSF animals that received tone-airpuff conditioning prior to the compound conditioning did not show any conditioned eyelink responses to the light. Subsequent light-airpuff training in this group indicates that there was no savings to the light. Animals that received PTX, however, showed reliable conditioned responses to the light as well as a marked savings. These results support the view that the inferior olive provides the unconditioned stimulus information to the cerebellum for classical eyeblink conditioning.

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652.3  

The delineation of neural circuits that mediate behavior provides a foundation for determining how neural plasticity might occur and how these changes might alter behavior. The acoustic startle reflex in the rat has proven to be extremely sensitive to the presence of drugs and is therefore being used as a model system to analyze behavioral plasticity in vertebrates.

Using a variety of lesion techniques, we have approached a primary acoustic startle circuit that consists of one of the auditory nerve, parvestibular cochlear nuclei (PCN), an area dorsal to the lateral nucleus of the inferior colliculus (VLL), parameatal zone (PLZ), and the retroposterior nucleus (RP) of the lateral superior olive. The lateral nucleus of the inferior colliculus, VLL, and the retroposterior nucleus (RP) show cell bodies and terminals in both the ipsilateral and contralateral PCN, respectively.

To investigate the functional significance of these connections more definitively, we are currently using biotin to code cell bodies of each of these structures (VLL, RP, PLZ) in the presence of the lesion. By marking the contralateral PCN using retrograde transport and anterograde transport, we will be able to analyze the RPO clearly labeled cell bodies in the contralateral PCN, and terminals in both ipsilateral and contralateral PCN, respectively.

The present studies attempted to identify more precisely the locus of this relay in the startle circuit.

652.5  
ELECTROLYTIC LESIONS OF THE AMYGDALA BLOCK ACQUISITION AND EXPRESSION OF CONDITIONED FEAR EVEN WITH EXTENSIVE TRAINING, BUT DO NOT PREVENT REACQUISITION, AS ASSESSIBLE BY FEAR-POTENTIATED STARTLE. M. Kent* & M. Davis. Dept. of Psychiatry, Yale University School of Medicine, 34 Park St., New Haven, CT 06508.

The amygdala is well-known to be important for aversive conditioning. Lesions of the amygdala block the expression of fear-potentiated startle in which the amygdalectomized rats are acutely (when stimulated in the presence of a cue previously paired with footshock) or habituated (20 min after initial exposure to a cue) when stimulated in the presence of a cue previously paired with footshock. However, it has not been determined how extensive training would alter this lesion effect.

In Exp. 1, we tested lesioned (jig:N rats paired with 0.6 mA footshock US) and control rats (jig:N US) on day 30. Control rats showed significant freezing by day 30, but lesioned rats did not. These results suggest that the amygdala is involved in the expression of fear-potentiated startle regardless of the degree of learning. Also, the amygdala is necessary for the acquisition of fear-potentiated startle and other brain structures do not support this form of learning when training occurs without the amygdala.

652.6  

Conditioning episodes can modify attentional processing of conditioned stimuli (CSs). Some of these changes can be characterized as increased attention to the CS, whereas others reflect reduced attention to the CS. Of course, these changes are not mutually exclusive.

Liang (1991), who reported that pre-retention test injections of lidocaine elicit ORs prior to conditioning. This work examined whether CN damage alters selected CS processing.

Our results indicate that CN lesioned rats fail to exhibit conditioning that depends on increments in CS processing. This deficiency was evident in failures to show the enhanced associability of a CS that is normally observed when either an inconsistent predictive relation is arranged between that CS and another cue, or the reinforcement value of the unconditioned stimulus (US) is reduced in an unblocking procedure. In contrast to these impairments, CN damage did not affect the reduction in associability of a CS produced by CS preexposure (latent inhibition), blocking, or consistent reinforcement procedures. Thus the CN appears to be part of a neural system that regulates broadly-based increments in processing or attending to signals for biologically significant events, but is not critical for tuning out redundant or uninformative cues. Supported by NIMH grant 35554 and a NIMH RSDA to MG (K02-MH0806).

652.7  

The central nucleus (ACe) and the lateral/basolateral complex (LBL) are two areas within the amygdala that have been extensively implicated in learning and memory. The present experiments are designed to examine the role of the ACe and LBL in the retention of inhibition avoidance by reversely inactivating these regions after training. Male Sprague-Dawley rats (175-200 g) were implanted bilaterally with cannulae aimed at the ACe (AP -0.25; ML ± 0.4; DV -0.5 cm) or the LBL (AP -0.31; ML ± 0.31; DV -0.5 cm). One week later, the rats were randomly assigned to one of two drug conditions: 0.1 mg/kg lidocaine was delivered into the cannulae via the saline vehicle (0.9% saline) at 45 min after the drug delivery, with vehicle control. Animals that received vehicle injections into the ACe did not differ from those that received injections into the LBL. However, animals that received injections into the ACe had significantly shorter escape latencies. This lesion procedure did not produce any spontaneous reductions in fear-potentiated startle. However, these results indicate that the LBL may be involved in regulating the consolidation of aversively-associated memory. This interpretation is in agreement with the findings of Lee et al. (1991), who reported that pre-retention test injections of lidocaine into the basolateral amygdala impair retention only when retention is tested 2 days after training, but not 21 days after training.

Supported by 1967 NSERC/Canada (to MBP) and USPHS grant M12256 from NIMH & NIDA & ONR N00-140-91-0162 (to JLM).
652.9


Lesions of the ventral portion of the periaqueductal gray (dPAG) reduce defensive freezing to conditional fear stimuli. The function of the more dorsal portions of this structure lateral to the aqueduct (vPAG) on conditional freezing is unclear. In order to investigate the role of these regions in mediating freezing, rats were given electrolytic lesions of either the vPAG or dPAG. There were two types of controls (sham & superior colliculus lesioned). The rats were given electric footshock immediately after placement in a chamber for 3 consecutive days. This procedure does not provide the animal with ample time to appreciate the contextual cues prior to shock and therefore does not normally condition defensive behavior. However, rats with dPAG lesions were unique in acquiring unconditioned freezing using this procedure. Following this treatment, all the rats received a shock after being given a 3 min period to explore the chamber. All rats, except those with vPAG lesions, exhibited a high level of conditional freezing. During several extinction tests, dPAG lesioned animals showed enhanced, and vPAG lesioned animals showed reduced, freezing. Superior colliculus lesioned animals never differed from shams. These data suggest that the vPAG mediates conditional freezing while the dPAG inhibits acquisition of this defensive behavior.

652.11


Previously, we reported that electrolytic lesions of the dorsal hippocampus, made shortly after Pavlovian fear conditioning, eliminated the fear response to contextual stimuli associated with shock. The present study sought to extend that finding to pre-conditioning elimination of cell bodies in that structure. We made excitotoxic lesions, which spare fibers of passage, prior to conditioning. Conditioning to contextual cues immediately after the first conditioning trial was not impaired in the lesioned animals. However, as training progressed, hippocampal animals showed markedly attenuated conditional responding. In a shock-free retention test given 24 hours later, hippocampal lesioned animals showed severe impairments in responding to contextual fear cues. This pattern of results clearly parallels the effects of competitive NMDA antagonists on contextual fear conditioning. In conclusion, hippocampal lesions made either before or after training reduce contextual fear and this effect appears to depend on neurons intrinsic to the hippocampal formation.

652.10

DORSOLATERAL PERIAQUEDUCTAL GRAY LESIONS AND BENZODIAZEPINE AGONISTS AND ANTAGONISTS DISASSOCIATE ASSOCIATIVE AND NONASSOCIATIVE FEAR CONDITIONING. J. P. DeCola* & M. S. Fanselow. Dept of Psychology, UCLA, Los Angeles, CA 90024.

Previously we demonstrated that administration of the NMDA antagonist APV, blocks associative fear conditioning but does not block the sensitization of conditional fear. The present experiments further dissociate associative and nonassociative fear conditioning. Sensitization of fear can be demonstrated behaviorally as an enhanced response to a conditional stimulus (CS) when conditioning is preceded by exposure to unconditional stimuli (US). In our paradigm, the sensitization pretreatment is done by exposing rats to a series of footshocks in a distinct context the day prior to a single pairing of a novel contextual CS, and a single shock US. The sensitized animals exhibited enhanced fear to the novel context as compared to a non-sensitized group. Lesions of the dorsolateral periaqueductal gray (dPAG) prior to the sensitization treatment resulted in an enhanced sensitization of fear to the novel context. Paradoxically, both the sensitization treatment and dPAG lesions produced a reduction in unconditioned responding (activity) to the shock. Administration of the benzodiazepine (BZD) agonist tetrazepam (20 mg/kg, i.p.) prior to the sensitization treatment in the first context did not affect the sensitization of fear observed in the second novel context. However, it did block the acquisition of conditional fear to the first context where the pretreatment shocks were delivered. The BZD antagonist Ro15-1788 (7mg/kg, i.p.) had no effect on conditional fear but tended to enhance the sensitization effect.

652.12


BXSB mice are autoimmune and have approximately a 50% incidence of colic ectopias. Because of associations between immune disorders, ectopias, and developmental learning disorders in humans, we have been investigating behavior in these mice. The autoimmune BXSB mice are not normally freezing and are more severe in males because of a Y-chromosome autoimmune accelerator gene. To determine the effect of this gene on behavior, BXSB mice with the autoimmune accelerator gene (BXSB-Yaa) were compared to BXSB mice lacking the gene (BXSB-Yaa+). The mice were tested on a behavioral battery. Blood samples were obtained for immune analyses and their brains were examined for ectopias. Significant differences between the two groups were not found for activity and learning measures. BXSB-Yaa+ were more active in a swimming rotation task, and performed better in discrimination learning and avoidance conditioning. BXSB-Yaa+ had better performance in water escape, the Lashley maze, and the Morris maze. Ectopias, in interaction with strain, sex, and paw preference, significantly affected learning measures. There was no difference in the incidence of ectopias between the two strains, although there was a significant sex difference with males having more ectopias (60% and 40% for females).

Strain differences in males might be expected since they differed genetically. Strain differences between the two female groups are indicative of genetic drift. Therefore, it is unlikely that the males differ only with respect to the Yaa gene. The reason for the sex difference in ectopia incidence is not known, but is not due to the autoimmune accelerator gene, since a higher incidence was also seen in the non-accelerated strain. This work was supported in part by NIH grant HD 20896.

LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—CONDITIONING III

653.1


General facilitation effect of the unconditioned stimulus (UCS) on the conditioned response has been reported in many studies. Here, more specific effect is shown: the time amplitude characteristics of evoked slow potential response in the conditioned stimulus (CS) period changed as a result of paired training similar to the waveform of the unconditioned response (UCR). A tone CS was given to one ear of the cat eliciting orienting head movements to the direction of the tone accompanied with evoked neural response in recorded brain sites. The brain stimulation UCS to the lateral hypothalamus elicited, in turn, a typical head movement and unconditioned evoked slow potential response pattern. Comparison of the unconditioned presentation of the CS and UCS suggests a significant correlation (between .46 and .95) was found to emerge as a result of paired conditioning. Three alternative explanations were examined: first, the observed similarity between the evoked neural UCR and developing conditioned response, using the interstimulus interval (ISI) might be results of a general, increased facilitation of the CS pathway; second, any strong stimulus such as an UCS might disclose some typical "maximum orienting response", specific to the tone CS; and third, the UCS may actually modify the CS-pathway system; it might leave a temporal "memory trace" on the time-amplitude course of the CS pathway system. The results presented here supported the "memory trace"-hypothesis.

653.2

FUNCTIONAL NEUROIMAGING OF CONDITIONED INHIBITION EFFECTS ON THE AUDITORY SYSTEM OF THE RAT: FLUORODEOXYGLUCOSE MAPPING AND STRUCTURAL MODELING. R. McIntosh* & R. McIntosh. Dept. of Psych and Instt of Neurosci, Univ of Texas, Austin, TX, 78712, USA.

[14C(UJ)-2-fluoro-2-deoxyglucose (FDG) was used for functional neuroimaging of the auditory system using an acoustic conditioning paradigm as either a conditioned excitatory (A+) or inhibitor (X-). Structural modeling (McIntosh & Gonzalez-Lima, Brain Res. 547, p. 295, 1991) used the mapping data to demonstrate interactions between auditory structures related to conditioning. Rats were trained in two phases. In the A+ phase, a tone was paired with footshock for Group 1 and a light was paired with footshock for Group 2. In the X- phase, reinforced trials of A+ were intermixed with non-reinforced trials of the tone-light compound (AX-). For Group 1, the tone was the excitor, for Group 2 the tone was the inhibitor. After conditioning, both groups were injected with FDG and presented with the same stimuli. Group 1 showed greater FDG uptake in the dorsal cochlear nucleus (DCN) and the external s. of the inferior colliculus. Structural models of the auditory system showed that effects through the direct lemniscal pathway were similar for both groups, but effects of lemniscal-adjacent paths were in opposite directions for the two groups. Extra-lemniscal inputs were stronger for Group 2 suggesting that magnitude of the differentiation of the strong and the weak effect is a function of alterations in the interactions between parallel auditory pathways. These data indicate that auditory pathways may code both the physical parameters of stimuli and the behavioral significance acquired through learning. (Supported by NIMH grant ROI 43353.)
LEARNING AND MEMORY: SYSTEMS AND FUNCTIONS—CONDITIONING III

unconditioned HR orienting response. In continued testing in a new

presentations of either an 87- or 120-dB white noise stimulus for 10 trials

central nucleus (ACE) disrupt both conditioned freezing and HR

elicited by acoustic stimuli in rats. B.J. Young* and R.N.

AMYGDALA CENTRAL NUCLEUS LESIONS ATTENUATE THE

unconditioned effect of chronic shock was observed only in the

insular, perirhinal, and hindlimb cortices. The relative unconditioned effects were small in part because of the larger effect of simply exposing the rat to the apparatus.

CONDITIONAL CONTROL OF FLUID CONSUMPTION BY DISCRIMINATIVE CUES IS INDEPENDENT OF THEIR PAVLOVIAN ASSOCIATIONS. G.M. Martin D.M. Skinner, A.

nuclei and auditory cortex. These effects were not simply a generalized

increase in activity in the conditioned rats, but involved a frequency-dependent


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653.3

SELECTIVE ACTIVATION OF THE CEREBRAL CORTEX OF RATS DURING CONDITIONED AND UNCONDITIONED STRESS—REVEALED BY THE IMMEDIATE EARLY GENE C-FOS. C. R. A. Beck* and H. C.

Fibiger. Department of Psychology, University of Alberta, Edmonton, AB, T6G 2R9 and Division of Neurosciences, Department of Psychiatry, University of British Columbia, Vancouver, BC, V6T 1S3.

Male hooded rats were individually shocked acutely (1 session), shocked chronically (3 sessions), or were placed in the shock box being shocked. With the multiple cues of the shock box serving as contextual cues, the rats were assigned to groups (n=7) as unconditioned or conditioned on the final session (conditioned shocked previously but not on the final session, or control animals. Two hours after the beginning of the final session, the animals were terminated, perfused, and their brains removed. Following immunohistochemistry, fos-positive nuclei were counted on coronal sections of the cerebral cortex. Exposure to conditioned cues, compared to being placed in the box without shock, resulted in increased fos counts in several allolocortical (piriform, infralimbic, cingulate, retrosplenial, lateral orbital, parahirnal, and entorhinal) and allocortical structures (see Tondover occipital, frontal area 2, forelial, and hindlimb).

surprisingly, the unconditioned effect of acute shock appeared only in the retrosplenial cortex. The unconditioned effect of chronic shock was observed only in the insular, perihinal, and hindlimb cortices. The relative unconditioned effects were small in part because of the larger effect of simply exposing the rat to the apparatus.

653.5

CONDITIONAL CONTROL OF FLUID CONSUMPTION BY DISCRIMINATIVE CUES IS INDEPENDENT OF THEIR PAVLOVIAN ASSOCIATIONS. G.M. Martin D.M. Skinner, A.

DISCOVERY OF PASSIVE AVOIDANCE AND MILK MAZE DEFICITS WITH DISCRETE LESIONS OF THE SUBSTANIA INOMINATA OR GLOBUS PALLIDUS. B.G. Meyer* and C.P.

Gonzalez-Lima1* & J. Agudo2.

653.6

DOUBLE DISSOCIATION OF PASSIVE AVOIDANCE AND MILK MAZE DEFICITS WITH DISCRETE LESIONS OF THE SUBSTANIA INOMINATA OR GLOBUS PALLIDUS. B.G. Meyer* and C.P.

Gonzalez-Lima1* & J. Agudo2.

653.7

AMYGDALA CENTRAL NUCLEUS LESIONS ATTENUATE THE BRADYCARDIA, TACHYCARDIA AND BEHAVIORAL FREEZING ELICITED BY ACOUSTIC STIMULI IN RATS. J.M. Leeson, Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

In a preliminary experiment phasic heart-rate (HR) responses of rats to a 120-dB startle stimulus were examined during habituation across trials, and accelerations which developed across trials in a manner that resembled the development of freezing behavior. A 92-dB stimulus evoked little freezing or tachycardia yet evoked decelerations of similar magnitude to the 120-dB stimulus. Since lesions of the amygdala central nucleus (AC) disrupt both conditioned freezing and HR responses, we studied the effects of ACE lesions in this startle-HR paradigm. Bilateral electrolytic lesions were made in one group of rats and another group served as sham-control. Subgroups received 10 presentations of either an 87- or 120-dB white noise stimulus for 10 trials (6-s interstimulus interval) on four consecutive days. As predicted, ACE lesions impaired the development of both HR and freezing responses. Groups SI and 1SI did not differ, but SI and 1SI froze more and were made to more conflicts with previous data which suggest no involvement of ACE in the unconditioned HR orienting response. In continued testing in a new context, for 5 and 10 trials at 87 and 120-dB, the ACE animals froze more and of a lesser magnitude than observed in rats exposed to unconditioned aversive stimulation, and manifested as the diminution of contact sensitivity response amplitude; freezing behavior correlated with immunosuppression.

653.8

ACOUSTIC STARTLE STIMULATION SUPPRESSES IMMUNOLOGICAL FUNCTIONING AS INDEXED BY A CONTACT SENSITIVITY RESPONSE. R. Bron & J. Cranney* and J. Young and R.N.

The present studies investigated the immunological effects of novelty, startle stimulation and context conditioning in adult male wistar rats. Behavioural indices of fear responding (freezing, grooming, defecation and exploratory behaviours) were measured on their immunosuppressive effects of startle stimulation could be conditioned behaviourally in the rat. This suppression was more transient and of a smaller magnitude than that observed in rats exposed to unconditioned aversive stimulation, and manifested as the diminution of contact sensitivity response amplitude; freezing behavior correlated with immunosuppression.

653.4


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We previously reported (Soc. Neurosci. Abstr., 17:480) that discrete electrolytic lesions along the rostral-caudal extent of the substantia innominata (SI) in the rat produced differential deficits in drinking passive avoidance (dPA), step-through (sPA), and milk maze (MM) performance. The present study examined the effects of bilateral lesions at three sites in the SI (placed relative to bregma): central (SI: A-1.4, L±3.0, V-5.6), lateral (L±1.4, L±3.0, V-8.4), and rostral (SI: A-0.7, L±2.5, V-8.4). Also, lesions were placed in the globus pallidus: central (GP): A-1.4, L±3.2, V-7.2, or rostral (rGP): A-0.7, L±2.7, V-7.0).

Significant deficits in dPA were produced by SI and also lesions, but not SI, GP, or rGP lesions. The control group (COM) required 23±2 (M±SE) foot shocks of increasing intensity to avoid drinking for 5 min. Groups SI and rSI had 38±2 and 35±3, and rSI, GP, and rGP required 24±2, 27±2, and 28±2 foot shocks, respectively. Parallel results were found for sPA. In contrast to PA behavior, MM performance was poor in Groups SI and GP, and markedly impaired in Group rGP. Particularly notable is that Groups SI and rSI, compared to Group rGP, were significantly worse in PA and better in MM performance: a double dissociation.
563.9

Two experiments examined the effects of ibotenic acid lesions of the central nucleus of the amygdala (CeA) and the dorsal, ventral and ventral (vPAG) periaudictal gray on the relationship between freezing responses and the sensitization of the acoustic startle response in adult male Wistar rats. CeA lesions attenuated both the non shock related sensitization of the startle and the sensitization of startle by shock, and attenuated post shock freezing and freezing elicited by shock associated cues. vPAG lesions attenuating freezing but failed to attenuate sensitization of startle, eliciting a tonic augmentation instead. vPAG lesions had no significant effect on either startle or freezing responses. These results support the hypothesis that the CeA constitutes part of the final common pathway of an integrated defensive response.

563.11

PKC is involved in neural plasticity, and phosphorylation of a PKC substrate (MARCKS) in part of the chick hyperstriatum (IMHV) has been shown to correlate significantly with the strength of learning in filial imprinting (McCabe et al. Soc. Neurosci. Ab. 17, 140). The distribution of PKC and PKCα,β,γ in the brain of day-old dark-reared chicks was determined immunocytochemically. Frozen coronal sections (20 μm) were incubated with M5S, or with 36G5, monoclonal mouse anti-PKCα and anti-PKCγ, respectively. PKCγ-stained cells were distributed widely in the telencephalon, incl. all hyperstriatal structures (incl. IMHV), the hippocampus (H), Neostriatum (N), Ectostriatum (E) and Archistriatum (A). There was less dense staining in the Septum (S), and the least cellular staining was in the Paleostriatal complex (P). The distribution of PKCα,β-stained cells was more limited, with staining in A, H and S but not in the hyperstriatum. However, there was PKCαβ staining of fibres in part of IMHV (but little elsewhere in hyperstriatum ventrale), in N, P and in Lobus parolfactorius. These results raise the possibility that PKCαβ may interact with the ACh system to play a role in the generation and expression of behavioral responses.

563.12
PARTICULAR SHOCK AVOIDANCE (PSA) INDUCES CHANGES IN PKC, MAP2, AND MUSCARINIC ACETYLCHOLINE RECEPTOR IMMUNOREACTIVITY IN SINGLE CORTICAL NEURONS. E.A. Van der Zee1, B.R.K. Bouma1, A.D. Sterenberg1, B. Bohus1, and P.G.M. Luiting1, Lab. Animal Phys., Univ. of Groningen, Haren, The Netherlands.

Cortical activity is enhanced during PSA. In experiment I changes in immunoreactivity (ir) for mAChRs, PKCγ and MAP2 after PSA were examined. 18 Young adult male Wistar rats were divided into 3 groups: naive controls (n=6), no shocked controls, introduced to the testapparatus (n=6), and shocked animals (n=6). 24 hr after the last trial, the animals were perfused. Cryosections were labeled for mAChRs (M3), PKCγ (36G9), and MAP2. In addition, immunocytochemistry was performed for mACRs (PKCγ), Cholinergic receptors often express PKCγ, and mAChRs appear to be highly colocalized with MAP2. Notably in the shocked group, the ir for all 3 markers was increased in individual neurons. These neurons were found in radial cortical columns, often in a lateralized fashion (either in left or right hemisphere). In experiment II we examined the contribution of ACh to the enhanced ir. Of 6 rats, the nucleus basalis magnocellularis was unilaterally lesioned. Cortical columns with enhanced ir were observed, both on the lesioned and nonlesioned side. However, clear deficits in PSA was observed when the columns overlapped with ACh depletion. The results reveal that 1) enhanced ir for mAChRs, MAP2 and PKCγ is found in single cortical neurons, 2) neuronal activation induced by PSA causes the changes in ir, 3) ACh is not required for this induction, but 4) learning deficits arise when the behaviorally activated neurons lack cholinergic innervation.
564.1 

Cephal (1982) showed that rats trained to run an alley for a large food reward displayed sharp and rapid latencies when shifted to a small reward. This effect is referred to as behavioral contrast and is usually interpreted as an aversive emotional reaction to a reduction in reward magnitude. We have shown that benzodiazepines (BDZZ) produce a characteristic decrease in latency on the second day of shift training. This effect is potentiated in animals receiving 1 mg/kg midazolam (MDZ) or saline. During the training session one half minutes prior to the next training session, half the animals were injected (i.p.) with 1 mg/kg midazolam (MDZ) or saline. Shifted animals receiving saline displayed a characteristic sharp increase in latency on the second day of shift training. This increase was not visible in the shift/MDZ animals on that day but it did appear on the third day. This study suggests that MDZ injected immediately prior to a reduction in reward magnitude impairs the retention of the aversive consequences of such a decrease.

Supported by NSF fellowship RCD-9054728 (JS) and PHS MH1526 (NIMH and NIDA) and ONR N00014-90-J-1626 (JLM).

564.2

It is well known that benzodiazepines (BDZ) induce anterograde amnesia in humans and laboratory animals. Recent findings suggest that the memory impairing effects of BDZ's involve the modulated complex (AC) of cholinergic cell bodies and therefore significantly confined the basal forebrain GABA-cholinergic link. 

Supported by PHS MH15256 (NIMH & NIDA) & ONR N000-14-90-J-1626 (JLM).
**654.7**


The effects of the benzodiazepine receptor (BZ) full agonist chlordiazepoxide (CDP) and midazolam, and the partial agonist t-carboline ZK 91 296 on the performance of rats in a simple reaction time paradigm were examined. This task required the animals to respond to rarely and unpredictably occurring brief (50 msec) visual stimuli. The dose-dependent effects of midazolam on signal sensitivity and general responsivity occurred in parallel. In contrast, the effects of CDP on signal sensitivity were largely independent from effects on response bias. The partial agonist ZK 91 296 in general had little effect on performance. Extension of the stimulus presentation time attenuated the effect of CDP on signal sensitivity. These results support the hypothesis that BZ agonist-induced disruption of attentional abilities is not necessarily confounded by effects on general responsivity or sedation, and thus may represent a discrete pharmacological property of these compounds. We are currently examining the hypothesis that CDP-induced impairment in behavioral vigilance depends on the integrity of the basal forebrain GABA-cholinergic link.

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**654.8**


Both clinical and experimental evidence have found systemic chlordiazepoxide (CDP) to reduce anxiety and impair new learning. Whether these two actions are causally related is unknown. This study sought to determine if different neuroanatomical regions mediate the anxiolytic effects of CDP on exploration (thigmotaxis) in an open field and the amnesic effects of CDP on place learning in the Morris water maze.

Firstly, rats received systemic (i.p.) injections of either CDP (5 mg/kg) or saline and tested for anxiety in the open field and amnesia in the water maze (1 d, 20 trials followed by probe; 1 min ITI; 15 s on platform; 45 s in warming cage). Systemic CDP suppressed thigmotaxis (reduced anxiety) and impaired place learning (produced amnesia). New rats were implanted with a cannula aimed at either the medial septum, or bilateral cannulae aimed at amygdala or frontal cortex. CDP (60 mmol) was dissolved in a 1 µl volume of ACSF and infused over a three minute period. Controls were infused with ACSF. Intra-amygdala infusions of CDP suppressed thigmotaxis but had little effect on place learning and intra-septal infusions of CDP had little effect on thigmotaxis but impaired place learning. Infusions of CDP into the frontal cortex had little effect on either thigmotaxis or place learning. These results suggest that the anxiolytic and amnesic actions of systemic CDP are mediated by the amygdala and medial septum, respectively. (Research Supported by B.C. Health Care Research Foundation and NSERC, Canada)

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**654.9**

**REVERSAL OF CHLORDIAZEPOXIDE-INDUCED IMPAIRMENT IN DISCRIMINATION PERFORMANCE BY PICOXOTIN.** S. O. Cole and J. V. Martin. Departments of Psychology and Biology, Rutgers University, Camden, NJ 08102.

The effects of chlordiazepoxide (CDP) alone and in combination with picrotoxin (PTX) on the performance of a previously-learned go-no go successive discrimination task were studied in male Sprague-Dawley rats. CDP 10 mg/kg impaired discrimination in five successive drug sessions, with animals demonstrating recovery in a single post-drug session. The impairment in discrimination performance was due to an increase in responding during the no go period of the task (errors of commission). The γ-aminobutyric acid (GABA) antagonist PTX (0.5, 1.0 mg/kg) reversed the CDP-induced impairment in discrimination performance and reduced the number of incorrect responses in a generally dose-dependent manner when co-administered with CDP.

Although the co-administration of CDP and PTX produced a significant reduction in responding during go periods of the task (which further reduced the overall response rate), this effect worked against an improvement in discrimination performance rather than contributing to it. When administered alone, PTX produced no significant change in discrimination performance. These findings suggest that the impairment in discrimination performance by CDP is mediated by actions on the central GABA, receptor-benzodiazepine receptor-chloride channel complex.

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**654.10**

**MEMORY DEFICITS RESULTING FROM DIENCEPHALIC DAMAGE IN MICE ARE REVERSED BY AN I.P. ADMINISTRATION OF METHYL β-CARBOXYLATE.** D. I. Beracochea (*1), A. Krazem (2). (1)Lab. Neurosciences Cognitives et Comportementales, CNRS URA 339, Univ. Bordeaux 1, Av. des Facultés,33405 Talence, France and (2) Université d Tizi-Ouzou, Algeria.

Diencephalic damage was induced either by a chronic (12-months) alcohol consumption (AC)(see Bonemtapi et al poster) or by experimental ibotenic acid lesions of the mammillary bodies (MB). Previous studies have shown that both AC and MB lesions decreased anxiety in the open-field and in the elevated-plus maze, and induced memory impairments in spatial alternation tasks. We hypothesized that hypoxia might be in part responsible for these deficits. Accordingly, increasing anxiety in experimental subjects should attenuate the memory impairments. Three doses of β-CCM (0.25, 0.5, and 1.0 mg.Kg−1) were used, according to previously determined anxiogenic properties. Results showed that the administration of β-CCM improved alternation rates in all groups, as compared to saline-treated subjects. However, the alternation deficits were reversed by a lower dose in the AC group (0.5mg.Kg−1) than in the MB group (1.0 mg.Kg−1). Since AC induced weaker lesions of the MB than a direct administration of ibotenic acid, one can conclude that part of the facilitative effect of β-CCM might be a function of the severity of damage to the mamillary system.
655.3

BEHAVIOURAL, BIOCHEMICAL AND HISTOLOGICAL EFFECTS OF TRIMETHYL-INDUCED BRAIN DAMAGE IN THE RAT

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The nature and extent of the behavioural, biochemical and histological changes induced by the organotin compound trimethyltin (TMT) was investigated in the Sprague-Dawley rat. TMT doses were treated with single injection of TMT (6.0, 7.0 or 8.0 mg/kg ip) and behavioural experiments were performed 24-28 days after treatment. Histological analysis of TMT administration was done dependent and included (a) hyperactivity in the open-field test, (b) increased locomotor activity in a narrow space, (c) tail-swatting, (d) some ataxia and Morris water maze performance. Post-mortem histological analysis revealed damage to hippocampal pyramidal cells, an effect which was particularly apparent at 8.0 mg/kg. After a single injection of TMT, HPLC and fluorimetric assays showed that 5-HT and GABA levels in the hippocampus and amygdala were decreased. Furthermore, using quantitative autoradiography, M1 and M2 receptor binding sites were found to be markedly diminished in the hippocampus. These results suggest that the toxic interaction of TMT with the hippocampus and other brain regions may be responsible for its detrimental effect on learning and memory.

655.5

MEMORY EFFECTS OF NEUROSTEROIDS INJECTED INTO THE NUCLEUS BASALIS MAGNOCELLULARIS OF THE RAT

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Since the discovery of marked cell loss and various pathological alterations in the nucleus basalis magnocellularis (NBM) in patients suffering from severe dementia of the Alzheimer type, this structure has been the subject of much attention. The NBM has been reported to be the main source of cortical cholinergic innervation. Various afferents of the NBM have been identified, and particularly a GABAergic afferent originating in the nucleus accumbens, that forms contacts with NBM cholinergic cells. GABA-A and benzodiazepine (BDZ) binding sites have been detected in the NBM. Functionally, local injections of GABAergic agonists into the NBM have been shown to block or reduce the activity of NBM cholinergic neurones. Behavioural studies using local injections of GABAergic agonists or antagonists into the NBM have been shown that this structure is involved in memory processes. Recently it has been demonstrated an interaction between endogenous steroids and the central GABA receptor. Steroids-GABA receptor interaction may have a role in the regulation of the NBM cortical projections. In order to test this hypothesis, we investigated the effects of local injection of pregnenolone sulfate (a GABA-like antagonist) and Tetra-Hydro-Pregnenolone (a GABA-like agonist) into the NBM on performance in a new two-trial memory task. Our results show that local injection of pregnenolone sulfate into the NBM improves memory performance in the Y maze recognition task. Conversely the injection of Tetra-Hydro-Pregnenolone decreases memory performance in this task. In conclusion, endogenous steroids can modulate memory processes and NBM-mediated dysfunctions. However, it remains to be elucidated if this effect results from an interaction at the GABA receptor level.

655.7

ACUTE ETHANOL DISRUPTS PATTERN CONDITIONING BUT NOT CONDITIONING IN ADULT AND ADOLESCENT ANIMALS. J. Rajendran, M.E. Speal and L.P. Speal, Dept of Psychology and Child Development, Pittsburgh State University, Pittsburg, KS 66555.

Effects of alcohol administration on conditioning to a visual stimulus and an olfactory context were examined in both adult (60-70 day old) and adolescent (35-38 day old) rats. Conditioning occurred in a patterned chamber divided into two compartments, one with vertical black and white alternating stripes and the other with horizontal, solid walls. Paired animals were given 5 training trials consisting of a 20 sec placement in the CS+ chamber followed by a 20 sec exposure to the CS- chamber, during which the rats were allowed to explore the compartments. Unpaired animals received the same regimen of footshock exposure in a black chamber 10 min prior to exposure to the patterned chamber and the orange odor. Ethanol (2.0g/kg) or saline was intragastrically administered 10 min prior to conditioning. Animals were given one of two 5 min preference tests after conditioning: either between the CS+ and CS- or between a novel saline odor and the orange odor. Ethanol did not disrupt conditioning to the odor context; at both ages paired animals pretreated with saline or alcohol exhibited an equivalent aversion to the orange odor. However, conditioning to the CS+ was significantly impaired by alcohol. While paired animals at both ages given alcohol did not acquire an aversion to the CS+, paired saline animals did learn the CS-US association. Thus it appears that a modest dose of alcohol impairs learning of the CS+ but not to the more general olfactory context in which the CS+ occurred.

655.8

PHARMACOLOGICAL MODULATION OF ACUTE-ETHANOL-INDUCED MEMORY BLACKOUTS IN RATS. D.H. Epstein and L.A. Pahlsberg, Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08855.

This study explored the neurochemical mechanisms of ethanol-induced amnesia. Behavioral interactions of ethanol (ET) with drugs whose actions are neurotransmitter-specific were evaluated in a paradigm that assessed the clinically relevant type of memory (episodic) under clinically relevant conditions (intoxication during acquisition). Male Long-Evans rats were trained in a Y-maze active-avoidance task whose episodic-memory component took the form of repeated reversals, each of which had to be remembered over a delay of up to 180 minutes. ET (25% v/v in saline, 2.5 g/kg IP) was shown to produce a dose-dependent deficit in memory consolidation, which were not dependent on deficits in acquisition or procedural memory. This ET-induced amnesia was partly prevented by coadministration of the anti-GABAergic drug Ro 5-5635 (5 mg/kg IP), mimicked and enhanced by GABA_A receptor activation with chlorodiazepoxide (1, 3, 5 mg/kg IP), and also mimicked and enhanced (but to a lesser degree) by general GABAergic augmentation with amnestic acid (15 mg/kg IP). This finding suggests that GABA_A receptors have a greater role than GABA_B receptors in ET-induced amnesia. Exposure of 3-H14 receptors with E-OH-DPAT (16.32, or 64 mg/kg IP) unexpectedly produced the most severe and behaviorally specific amnesia of any of the treatments, but the interaction of 8-OH-DPAT with ET, and the two drug-differing interactions with a 3-H14 antagonist [I-propargylol, 8 g/kg IP], ruled out H14 activation as the mechanism of ET-induced amnesia. The significant but not complete prevention of blackouts by GABA agonist suggests the involvement of other transmitter systems.
655.9  STAGES OF MEMORY FORMATION IN 2 AREAS OF CHICK BRAIN. M.R. Rosenzweig*, D.W. Lee, S.S. Shweder & E.L. Bennett. Dept. of Psychology, Univ. of California, Berkeley, CA. 94720, USA.

Two-day-old chicks were given bilateral i.c. injections into the intermediate medial hyperstriatrum ventrale (IMHV), trained on a 1-trial peck avoidance task using a target bead dipped in methyl anthranilate (MeA) then tested from 10 s to 24 h posttraining. After strong training (100% MeA), memory formation is impaired by 5 min posttraining by glutamate (GLUT, a long-term memory inhibitor), by 20 min by ouabain or scopolamine (OUAB or SCOP, intermediate-term memory inhibitors), and by 90 min by anisomycin (ANI, a long-term memory inhibitor). Memory for weak training (10% MeA) is also impaired by GLUT and ANI at the same doses and times as for strong training. Neither ITM inhibitor performed as predicted: OUAB impaired weak memory only at a dose that resulted in severe side effects, and SCOP inhibited weak memory at 24 h only. In contrast, injections of OUAB into lobus parolfactorius (LPO) resulted in significant memory impairment at the same doses and times as for strong training. Supported by NSF grant BNS-88-10528 & NIDA grants DA04795 and DA05396.

655.10  SEASONAL MODULATION OF LEARNING AND MEMORY IN CHICKS. D.W.Lee*, G.G. Murphy, E.L. Bennett, & M.R. Rosenzweig. Dept. of Psychology, Univ. of California, Berkeley, CA. 94720, USA.

Two-day-old chicks were trained on a 1-trial peck avoidance task using a target bead dipped in either 5, 10, or 100% methyl anthranilate (MeA). Groups were tested at times from 10 s to 24 h posttraining. Runs were conducted during 2 summers (Jun-Aug) and winter (Nov-Jan). At every test time, groups of chicks trained with 100% MeA showed the highest avoidance, followed by 10%, then 5% MeA; retention is dependent upon strength of training.

Winter chicks did not differ from summer chicks during training but showed less retention at test. They were also heavier and more active, but neither measure correlated with test performance. Performance for each strength of training was positively correlated to daylength.

In summer, test performance averaged over all 3 training strengths showed retention deficits ("dips") at 1', 15', and 60' test times. In winter, dips were present at 1'-5', 30', and 90' test times - thus winter not only impairs but also slows down memory formation on this task.

Supported by NSF grant BNS-88-10528 & NIDA grants DA04795 and DA05396.


The three stage model of memory formation proposed by Gibbs and Ng (1997) and extended by results from our laboratory was used to determine the role of protein kinase (PK) activity during memory formation. Groups of chicks were trained on a 1-trial peck-avoidance task 5 min after systemic agents were injected bilaterally into the intermediate medial hyperstriatum ventrale (IMHV). The time of appearance of amnesia induced by these PK inhibitors indicates the stage of memory formation disrupted by each agent. Our results show that intermediate-term memory (ITM) formation can be disrupted by agents affecting calcium or CAM kinase activity (e.g., W-9, W-13, OA-1004, TFP, A-3). Long-term memory formation can be disrupted by inhibiting PKA, PKC or PKG by agents such as H-7, H-9, H-9, ML-9 and HA-156; these agents had no effect on ITM. W-9, TFP, H-7 and HA-156 produced amnesia when injected into the left IMHV but not when injected into the right IMHV. Supported by NSF grant BNS-88-10528.


Electrophysiological and morphological data suggest that protein kinase C (PKC) is implicated in several forms of neuronal plasticity. Thus it has been proposed that activation of PKC and phosphorylation of particular substrates are critical events in the neuronal plasticity leading to an organic support of memory traces. In accordance with this statement, we have previously shown that spatial learning induced a decrease in cytosolic PKC activity in the hippocampus (NOUZUES et al., 1990, Soc. Neurosci. Abst., Vol. 16, 316-141). In order to investigate the functional aspects of these modifications, we have compared the mnemonic effects of intrahippocampal injections of either polymyxine B (PMB, 10 mM) or 2% lidocaine (0.2 µl bilaterally) on a mixed reference working memory task in a radial maze. When injected 15 minutes before each daily session, PMB was shown to slow down acquisition rate of reference memory. However, in contrast with all other groups PMB-treated animals exhibited forgetting on a retention test performed 16 days following acquisition. The beneficial influence of PMB treatment on forgetting might be explained by a post-acquisition rebound effect on PKC produced by the cessation of the PMB treatment. An alternative hypothesis (i.e. the induction of a direct extrahippocampal long-term storage) is currently under investigation.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
The effects of cocaine. A drug of abuse. A behavioral paradigm of place preference was used to examine the effects of the interstimulus interval (I5) on conditioning, with the chamber cues acting as the conditioned stimulus (CS) and intravenous cocaine as the unconditioned stimulus (US). The behavioral paradigm of place preference was used to examine the effects of the interstimulus interval (I5) on conditioning, with the chamber cues acting as the conditioned stimulus (CS) and intravenous cocaine as the unconditioned stimulus (US). The behavioral paradigm of place preference was used to examine the effects of the interstimulus interval (I5) on conditioning, with the chamber cues acting as the conditioned stimulus (CS) and intravenous cocaine as the unconditioned stimulus (US). The behavioral paradigm of place preference was used to examine the effects of the interstimulus interval (I5) on conditioning, with the chamber cues acting as the conditioned stimulus (CS) and intravenous cocaine as the unconditioned stimulus (US).
656.7

PAVLOVIAN CONDITIONING OF HYPERTHERMIA BY COCAINE. J. Broadbent*, J. F. Bachtold and O. L. Cunningham. Medical Psychology, Oregon Health Sciences University, Portland, OR 97201

The present study examined unconditioned and conditioned thermal responses to repeated infusions of cocaine. Rats implanted with an i.v. catheter and a biotelemetry device which measured core body temperature, were housed in cages enclosed in sound-attenuating chambers, and subjected to 16-hr sessions. Each session consisted of two 30-min trials. Subjects were presented with a light/noise cue (CS) for 30 min during the first trial; 15 min after the start of the CS, an i.v. infusion of 2 mg/kg of cocaine was administered to subjects. 'Unpaired' subjects received an identical cocaine infusion in the absence of the CS, 3.5 hrs later. 'Paired' animals did not receive any programmed events during this second trial. Initial infusions of cocaine produced little change in body temperature. However, with repeated exposure, cocaine was found to produce a mild hyperthermia in both groups. An anticipatory hyperthermia also became evident in the 'Paired' group following 20 conditioning sessions. This conditioned hyperthermia did not appear to summate with the thermal response to cocaine. Thus, in contrast to Pavlovian conditioning of thermal responses by ethanol and morphine, the cocaine-induced thermal conditioned response did not appear to mediate either tolerance or sensitization. Since previous data suggest that conditioned thermal responses alter drug self-administration, the present findings raise the possibility that conditioned response may also play a role in cocaine self-administration. (Supported by NIDA grant DA03608.)

656.8

TASTE REACTIVITY RESPONSES ELICITED BY COCAINE, PHENCYCLIDINE AND METHAMPHETAMINE PAIRED SUCCROSE SOLUTION. L.A. Parker*, Dept. of Psychology, Wilfrid Laurier Univ., Waterloo, ON, N2L 3C5, CANADA.

The nature of flavor-drug associations produced by a range of doses of the reinforcing agents cocaine (5 - 40 mg/kg, sc), phencyclidine (.5 - 20 mg/kg, sc) and methamphetamine (2 - 10 mg/kg, ip) were assessed by the taste reactivity (TR) test and the conditioned taste avoidance (CTA) test. At the highest doses tested, none of the agents produced aversive TR responses. At doses that produced equivalent strength of CTA, lithium did establish aversive TR responses. These results provide evidence that aversive TR responses are only produced by non-reinforcing drugs.

656.9

ELECTROPHYSIOLOGICAL RECORDINGS OF NUCLEUS ACCUMBENS NEURONAL ACTIVITY DURING A MODIFIED FR-3 SCHEDULE FOR COCAINE SELF-ADMINISTRATION. D.J. Woodward*, J.Y. Chang and S.F. Sawyer. Department of Physiology & Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27157.

We have previously demonstrated that neurons within the core region of the nucleus accumbens (NAc) exhibit anticipatory responses during cocaine self-administration bar pressing behavior. The purpose of the current study was to examine further correlations between neuronal activity in NAc and goal-directed behavior by using a modified fixed ratio 3 (FR-3) schedule to determine if the sequential bar pressing associated with different cues and consequence would produce different neuronal responses in NAc. A bundle of eight 2um diameter teflon coated stainless steel microelectrodes were chronically implanted in the NAc for extracellular single unit recording. After recovery from surgery, rats were trained to self-administer cocaine with the modified FR-3 schedule. A retractable bar was mounted on one side of the conditioning chamber. The first bar press resulted in bar retraction; the second bar press turned on the light and retracted the bar; the third bar press turned off the light and delivered cocaine (1 mg/kg). A two delay second delay was imposed between each bar press pressing, whether it led to cocaine delivery or not. In another experiment, in which a FR-1 schedule was associated with or without cocaine injection, the anticipatory response also remained essentially the same during extinction. These results suggest that conditioned neuronal responses expressed in the NAc are likely related more to a general goal-directed motoric behavior towards an object with significance rather than a reward-specific phase of the behavior. Supported by DA102338, and MH44339.

656.10

COCAINE-INDUCED CONDITIONED PLACE PREFERENCE IN RHESUS MONKEYS. S.M. Pomerantz*, L. Wertz, B. Hevner, L. Waslo, J. Piazza. Dept. of Physiology and Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Although numerous studies in rats have used a conditioned place preference (CPP) paradigm to assess the reinforcing actions of drugs, no studies have yet to demonstrate whether such a procedure can be employed in primate species. The aim of the present study was to develop a CPP paradigm in rhesus monkeys and evaluate the ability of cocaine to produce a CPP. A U-shaped test apparatus was designed comprising of a central compartment and two end compartments with colored side and floor panels. The experiment was run in three phases (Pretest, Drug Conditioning, and Posttest). In Pre- and Posttests, monkeys were able to explore the entire test apparatus for 30 min, whereas during conditioning, monkeys were restricted to one or the other end compartments for 30 min. Conditioning lasted 8 days. On odd-numbered training days monkeys were injected with cocaine (IM) and placed in the originally less-preferred compartment and on even-numbered days they were injected with saline and placed in the opposite, initially more-preferred compartment. The effect of 0.5, 50, and 100 µg/kg of cocaine (N=4) and 400 and 800 µg/kg of cocaine (N=4) on the acquisition of a CPP has been studied. Compared to their pretest, after being conditioned in one of the compartments with 0.5, 50, 100, 400 and 800 µg/kg of cocaine, monkeys spent on average 5.32, 45.6, 91.2±25, 113±20, and 80±40 more 5-sec blocks in the cocaine-paired compartment, respectively. Moreover, since the increased time in the cocaine-paired compartment was also associated with decreased time in the saline-paired compartment a reversal in place preference was observed. These data demonstrate that a CPP procedure can be used in rhesus monkeys to investigate the reinforcing properties of drugs.

656.11


Rats traversing as straight-alley for positive reinforcement typically exhibit faster running times, or goal latencies (GL), as training proceeds. However, when reinforced with cocaine, we observed that subjects took progressively longer to enter the goal box over trials. Closer observation of the data revealed that the increasing GLs were the result of a unique "retreat behavior" (i.e., stopping and returning to the start box). We hypothesized that such behavior reflected an inherent "conflict" that stemmed from the drug's well documented reinforcing and anxiogenic properties. To test this idea, the runway behavior of animals experiencing other concurrent positive and negative stimuli (i.e., food and mild footshock) was examined. Hunger, animals were trained to bar press for a runway for food reinforcement coupled with footshock. These subjects showed higher GLs and retreat frequencies than a control group which received only food in the runway. The nature of the pattern of the retreat behavior and the food+shock group strongly resembled that of cocaine-reinforced rats.

656.12


The classical conditioning of cocaine's behavioral effects with specific environmental stimuli is an important aspect of its actions. This property of cocaine is of major significance with respect to this drug's abuse potential, as intense craving can be evoked by stimuli previously associated with drug taking. To better understand the neuronal correlates of this phenomenon, the expression of the putative metabolic marker c-Fos and locomotor behavior were examined in rats that were exposed to an environment in which they had previously received cocaine.

Comparison to saline treated controls,acute administration of cocaine produced an increase in locomotor behaviour that was accompanied by an increase in c-Fos expression within specific limbic regions (cingulate cortex, caudaturn, lateral septum, paraventricular nucleus of the thalamus, and amygdala) as well as the basal ganglia (dorsomedial striatum and nucleus accumbens). Exposure of rats to the cocaine-paired environment also produced an increase in locomotion, as compared to pseudoconditioned and control subjects. In contrast to this behavioral effect, conditioned subjects exhibited a significant increase in c-Fos expression within the cingulate cortex, caudaturn, paraventricular nucleus of the thalamus, lateral habenula and the amygdala, suggesting an increased neuronal activity within these regions. In contrast, the dramatic effects observed within these limbic structures were not observed within the nucleus accumbens or dorsal striatum. The present findings suggest that specific limbic regions exhibit increased neural activation during the presentation of cocaine-paired cues and may be involved in the formation of associations between cocaine's stimulant actions and the environment in which the drug administration occurred.
565.13 THE 5HT1A RECEPTOR: RELATIONSHIP TO IMPULSIVE AND AGGRESSIVE BEHAVIOR IN COCAINE ABUSE.
Frederick G. Noeller*, Joel L. Steinberg, Ronald Cherek, Frederick Feifel, Larence Phillips, David Gerber. University of Texas Southwest Medical School, 4500 S. Lancaster, Dallas, TX 75216.

The neurotransmitter serotonin is linked to aggressive behavior in animals and humans. In order to examine the role of 5HT in aggressive and impulsive behavior in cocaine abuse, the 5HT1a agonist buspirone (0.4mg/kg orally) was administered as a neuroendocrine challenge agent to 7 cocaine dependent male human subjects, and 9 healthy male controls. The ACTH, growth hormone and prolactin levels after buspirone administration were then compared between the healthy controls and the cocaine dependent patients. Hormone levels were also correlated with measures of aggression including the Buss-Durkee Hostility Inventory. Buspirone levels were obtained on all subjects to control for differences in metabolism between groups. Results of this analysis will be presented, and the implications for treatment of aggressive behavior in cocaine dependent patients will be discussed.

565.14 EFFECTS OF INTRA-ACCUMBENS AND INTRA-PREFRONTAL CORTEX COCAINE INFUSIONS ON SCHEDULE-INDUCED POLYDIPSIA.

Schedule-induced polydipsia (SSIP), the excessive drinking induced by exposure to intermittent schedules of food-delivery, has been functionally linked to forebrain dopamine projections and in particular to the mesolimbic dopamine system. This study compared the effects of cocaine microinfusions into the nucleus accumbens (NACC), the medial prefrontal cortex (MPFC), and IP cocaine injections on schedule-induced drinking, locomotor activity and panel presses to gain access to the food.

SSIP was induced in male Wistar rats reduced to 85% of their free-feeding weight and exposed to a fixed-time 60 second schedule of food-presentation. When performance reached stable levels subjects were bilaterally infused with either vehicle, 12.5, 25, 50, or 100 ug cocaine HCI via chronically implanted guide cannula aimed to give access to either the NACC (n=12) or MPFC (n=12). The sequence of infusions was according to a Latin square design and infusions were administered immediately before the daily 30 min sessions. Following the series of intra-cranial infusions all subjects received IP injections of either saline, 2.5, 5, 10, or 20 mg/kg cocaine HCI.

The 3 routes of drug administration produced different profiles of behavioral effects. For example, both IP and NACC cocaine dose-dependently decreasedSSIP and increased locomotor activity suggesting a reduction inSSIP through response competition. However, MPFC cocaine also dose-dependently decreasedSSIP but did not significantly affect locomotor activity. In addition, IP cocaine increased low rates of panel pressing without affecting high rates whereas NACC and MPFC cocaine had no effect on low rates but decreased high rates of responding.

565.15 A PHARMACOLOGICAL INVESTIGATION OF GBR 12909-INDUCED BEHAVIORAL SENSITIZATION. B.A. Baldoc*, C. C. Hinton and A. E. Kelley. Department of Psychology, Northeastern University, Boston, MA 02155.

The present study was designed to investigate the mechanisms underlying GBR 12909-induced behavioral sensitization. GBR 12909, a psychomotor stimulant which selectively inhibits dopamine reuptake, was repeatedly administered to male Sprague-Dawley rats. Specifically, chronic treatment comprised of intraperitoneal injections of 20 mg/kg GBR 12909 or vehicle, administered every other day over a 12-day interval. Following cessation of chronic treatment, the animals were challenged with vehicle, GBR 12909 (6 mg/kg), and the following drugs, which were tested in separate experiments: amphetamine (0.1 mg/kg), nomifensine (0.75 mg/kg, 1.5 mg/kg), d-amphetamine (0.25 mg/kg, 0.75 mg/kg), bupropion (20 mg/kg), and scopalamine (0.1 mg/kg, 0.5 mg/kg). The dependent variable was locomotor activity, measured in photocell testing cages. Results show that animals chronically treated with GBR 12909 display a potentiated locomotor response to the 6 mg/kg GBR 12909 challenge, compared with their vehicle-pretrained counterparts. In addition, GBR 12909-sensitized rats display a hypersensitivity to other drugs which act at the dopamine uptake site: nomifensine, d-amphetamine, and bupropion. However, cross-sensitization to amphetamine, a direct dopamine receptor agonist, or to scopalamine, a cholinergic muscarinic antagonist, was not observed. These results are discussed with regard to possible presynaptic changes on dopamine neurons which may underlie behavioral sensitization to cocaine-like drugs.

565.16 COMPARISONS BETWEEN Dopamine UPTAKE BLOCKERS IN RATS TRAINED TO DISCRIMINATE COCAINE OR BUPROPION.
P.Terry* and J.L. Katz. Psychobiology Laboratory, NIDA Addiction Research Center, P.O. Box 5180, Baltimore MD 21224.

Bupropion (Wellbutrin) is a novel, non-tricyclic antidepressant; it is a weak inhibitor of dopamine uptake, and of several ligands to the dopamine transporter. Although its neurochemical and behavioral profile in vivo resembles that of a psychomotor stimulant, bupropion does not reliably produce stimulant effects in humans. This experiment examined and compared the discriminative stimulus effects of bupropion and cocaine in rats. One group of rats was trained to press one lever when injected IP with cocaine (10.0 mg/kg), and another lever when injected with saline; a second group of rats was trained similarly, but with bupropion (17.0 mg/kg) instead of cocaine. In substitution tests, full dose-response curves were obtained for several monoamine uptake inhibitors. In both cocaine- and bupropion-trained rats all nine dopamine uptake blockers tested to date fully substituted for the training compound. Across a 20-fold range, ED50 values in both conditions were highly correlated (r=0.81; p<0.05). Serotonin and noradrenaline uptake blockers failed to substitute even partially for either training compound. The results demonstrate a surprising similarity between cocaine and bupropion in terms of discriminative stimulus effects, and given the limited abuse of bupropion, suggest that this compound deserves further study as a potential therapeutic agent in cocaine addiction.


Two noncontingent footshocks (700 µA, 1 sec) were administered to rats in a dark compartment of a two-compartment, one-way avoidance chamber. Twenty-four hr later cocaine or saline was administered 5 min prior to a 30 sec re-exposure to selected stimuli present during the initial conditioning trials. On Day 3 the rats were trained to move from the chamber's dark compartment to the light compartment in order to avoid a footshock. Intermediate (5.0 or 7.5 mg/kg IP), but not low (3.3 mg/kg IP) or high (11.25 or 16.88 mg/kg IP), doses of cocaine given on Day 2 enhanced acquisition on Day 3 of the avoidance response. These results suggest that cocaine administered prior to the reactivation of cues associated with a conditioning episode can modulate memory processes, and that the dose-response function for this effect is U-shaped.

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The curve-shift rate-frequency paradigm was used to assess the ability of cocaine to potentiate the rewarding impact of lateral hypothalamic brain stimulation. In Experiment 1 animals (n=7) received daily saline or cocaine (0.5, 1, 2, 4, 8, 16, and 32 mg/kg i.p.) in ascending dose order. Cocaine produced dose orderly parallel leftward shifts of the rate-frequency functions; cocaine lowered the “dose” of brain stimulation required to produce normal responding. The highest dose shifted the rate-frequency functions by 0.4 log units thereby reducing self-stimulation thresholds by approximately 60%. The aim of Experiment 2 was to determine whether repeated testing (as in Experiment 1) might progressively alter the effectiveness of cocaine. A new group of animals (n=6) was treated 5 times with cocaine (16 mg/kg i.p.) at 48-h intervals. The magnitude of the reward-potentiating effect of cocaine did not change from treatment to treatment; there was neither tolerance nor sensitization to cocaine’s reward-potentiating action.
657.1  
DIFFERENT GENOTYPE-DEPENDENT FACTORS MODULATE SENSITIVITY TO THE BEHAVIORAL EFFECTS OF AMPHETAMINE. S. Cebrián*, A. Betancat, S. Puglis-Allegue. Inst Psicobiologia e Psicofarmacologia (CNR), via Reno 1,Roma I-00184, Italy

The existence of robust strain differences in the effects of chronic amphetamine treatment suggests that genetic factors influence behavioral sensitization processes. However, this phenomenon is produced by different experimental paradigms either involving sensitization or conditioned place preferenece and also by chronic or repeated exposure to environmental stress. Thus, the question arises as to whether similar strain differences are revealed by the different situations. DBA/2 are less sensitive to the stimulatory effects of amphetamine on locomotion than C57BL/6, although they are more susceptible to behavioral sensitization induced by repeated amphetamine injections. In the present study, however, the main result was observed in either strain of mice tested 24 hrs after the end of the treatment and C57BL/6 showed a rapid and robust sensitization when repeated psychostimulant injections were paired with the test environment. Following ten days of daily restraint sensitization developed to the behavioral effects of amphetamine in DBA/2 but not in C57BL/6 mice tested 24 hrs after the last stressful experience. Results obtained in B6D2F1 hybrids suggested that the response to repeated stress of the C57BL/6 strain is inherited through a dominant mode of inheritance. Finally, 7 days after the last stressful experience no sign of behavioral sensitization could be detected in this strain of mice.

These results indicate that behavioral sensitivity to amphetamine depends on an interaction between genotype-dependent factors and environment. Moreover, sensitivity to the behavioral effects of amphetamine does not predict susceptibility to either stress- or amphetamine-induced sensitization. Finally, such treatment capable of inducing sensitization produces different strain-dependent responses to amphetamine.

657.2  
INDIVIDUAL DIFFERENCES IN FEEDING CAN PREDICT INDIVIDUAL DIFFERENCES IN THE LOCOMOTOR RESPONSE TO AMPHETAMINE. T. L. Sills* & F. J. Vaccarino1,2. Departments of 1Psychology and 2Psychiatry, University of Toronto, Toronto, Ont., M5S 1A1.

Previously we demonstrated individual differences in the feeding response to a low dose of amphetamine (AMP); AMP stimulated intake in low baseline feeders and inhibited intake in high baseline feeders. Individual differences in the locomotor response to AMP, as a function of baseline activity, have also been reported. Intrinsic variation in nucleus accumbens dopamine activity has been suggested to underlie individual differences in both feeding and locomotion. In light of the feeding/locomotor parallels, the present experiment examined whether individual differences in baseline feeding would be predictive of individual differences in the locomotor response to AMP.

Thirty-four male Wistar rats (Charles River, Quebec) were divided into low and high feeders based on a median split of their sugar intake in response to 0.9% saline administration (ip). The locomotor response of each group to both a novel environment and a 1.75 mg/kg dose of AMP (ip) was subsequently determined. Results indicate that low feeders were significantly less active in response to a novel environment than high feeders. Similarly, low feeders exhibited significantly less locomotion in response to AMP than high feeders. These results are consistent with previous observations of individual differences in the locomotor response to AMP. Further, these results are consistent with the notion that individual differences in both the feeding and the locomotor response to AMP reflect intrinsic variation in a common substrate.

This research was supported by a NSERC grant to FJV.

657.3  
INFLUENCE OF SITUATIONAL VARIABLES ON THE EXPRESSION OF BEHAVIORAL SENSITIZATION TO AMPHETAMINE. S.J. Ahmed, L. Simon, M. de La Motte, University of Quebec, Quebec City, QC, Canada.

Behavioral sensitization is a progressive and long lasting enhancement to the psychostimulant effects of amphetamine (AMP). AMP-induced locomotor sensitization in rats has been shown that the expression of this phenomenon is under situational control. However, the two sets of contextual stimuli used in this paradigm for an association with the presence/absence of the unconditioned stimulus (US) is the unconditioned equivalent predictability value. In the present study, this possible bias has been circumvented by using the following protocol: two very distinct situational contexts both driven by the response of the unconditioned stimulus (US) usually is no more than a US present. Thus, the main results are: 1) the paired group (rats trained in the situational context paired with AMP) showedbehavioral sensitization compared to the control group (rats that have received the vehicle in the two contexts). However, the unpaired group (rats trained in the situational context paired with vehicle) failed to exhibit behavioral sensitization 2) when comparing the unpaired group with the control group, a great variability between rats appeared, some rats showed a response similar to the control group whereas other animals showed a response below the control group. In this latter subgroup, the influence of the situation appears to be more than an inhibition of the conditioned responses, since AMP-induced locomotor sensitization in AMPH has a reduction in unconditioned effects. This variability can be explained either by a difference in the sensitization process or a difference in the capacity to index context. Our preliminary results indicate that this variability could reflect a difference in the capacity of contextual indexation between animals.

657.4  
The EFFECTS OF CROWDING ON LOCOMOTOR ACTIVITY FOLLOWING CHRONIC METHAMPHETAMINE ADMINISTRATION. M. Blackshear* and B. Peoples. Department Of Biological Sciences, Tennessee State University, Nashville, TN 37209-1561

This study compares the effects of chronic methamphetamine administration on locomotor activity in isolated and crowded mice, and examines the effects of crowding on methamphetamine-induced "reverse tolerance". Swiss ICR male mice (26-30g) were used as experimental animals. The animals were either singly housed with 562cm² floor space/mouse or crowded with only 50cm² floor space/mouse throughout the duration of the study. Methamphetamine was administered at a dose of 4mg/kg. For the acute studies, the mice received a single injection of methamphetamine, while in the chronic studies, methamphetamine was administered daily for 7 days. Locomotor activity was monitored immediately after drug administration on day 1 (acute studies) and again (on day 9) at 48 hours after the last dose following a challenge dose of 1mg/kg of methamphetamine. Control animals were housed in a similar manner and received physiological saline. Predominantly, methamphetamine induced locomotor activity was higher on day 9 than on day 1 in mice that were singly housed (5.83 ± 7.78 vs 5.74 ± 5.25, respectively for days 1 and 9). In both instances, locomotor activity was higher in the drug treated animals than in the controls. In contrast, methamphetamine-induced changes in locomotor activity in crowded mice were essentially the same on day 1 and day 9 (5.98 ± 9.74 vs 5.53 ± 2.81 for day 1 and day 9, respectively) and suggests the development of tolerance, rather than reverse tolerance. Considering that reverse tolerance is thought to be a form of receptor sensitivity that is mediated by dopamine release, these findings suggest that the effects of crowding may precipitate changes in dopamine receptor sensitivity.

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657.5  
DIFFERENTIAL EFFECTS OF NUCLEUS ACCUMBENS LESIONS ON THE CONDITIONED PLACE PREFERENCE INDUCED BY MORPHINE OR AMPHETAMINE. M. C. Claussens* and K. B. L. Franklin. Dept. Psychology, McGill University, Montreal, Canada, H3A 1B1

It has been suggested that the mesolimbic dopamine system, originating in the ventral tegmental area and projecting to the nucleus accumbens (NAs) is involved in the reinforcing effects of both psychomotor stimulants and opioids. The post synaptic elements of this system have been shown to be a form of receptor sensitivity that is mediated by dopamine release, and also by intrinsic variation in a common substrate. Excitotoxin lesions were produced by bilateral microinjections of kainic acid (0.5 μg in 1 μl) into the NAs. The following recovery, different groups or rats were tested for the development of a CPP for morphine (2 mg/kg X 3 pairings) or amphetamine (1 mg/kg X 3 pairings). In both experiments, sham lesioned animals developed a CPP for the drug paired environment. Lesioned animals also developed a CPP for the morphine paired environment. Lesioned animals conditioned with amphetamine, however, did not show a significant preference for the drug paired versus the saline paired environment. These results support the suggestion that the reinforcing effects of psychomotor stimulants and opioids do not involve identical neural substrates.

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FRIDAY AM
**657.1**

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) INHIBITS GLUTAMATE-EVOKED FIRING OF NUCLEUS ACCUMBENS CELLS. S.R. White* and K.C. Paros, Dept. of VCAPP, Washington State Univ., Pullman, WA 99164.

MDMA is a recreationally used amphetamine derivative that has been reported to release serotonin (5HT) and, less potently, dopamine (DA) from nerve terminals in the forebrain (Schmidt et al., Biochem. Pharmacol. 26, 1977, 747). The nucleus accumbens (NAc), a major component of the brain reward pathway, is innervated by both 5HT- and DA-containing nerve terminals. This study examined the effects of local application of MDMA on excitability of NAc neurons in vitro. Extracellular single unit recording was combined with microiontophoretic drug application in urethane-anesthetized male rats. NAc cells were driven at a slow, stable firing rate by cycled pulses of glutamate. Microiontophoretic application of MDMA (10-40 nA, 60 sec) produced a slowly developing, dose-dependent inhibition of glutamate-evoked firing that was not blocked by administration of equivalent currents applied to a pH control solution. Application of 5HT and of DA produced a dose-dependent inhibition of glutamate-evoked firing that was similar to the effect of MDMA. The nonselective DA antagonist haloperidol partially attenuated this effect. These data suggest that MDMA-induced inhibition of NAc neuronal excitability may be mediated by both DA and 5HT receptors.

**657.2**

THE NONCOMPETITIVE NMDA ANTAGONIST MK-801 FAILS TO BLOCK THE REWARDING OR LOCOMOTOR-ACTIVATING EFFECTS OF AMPHETAMINE IN RATS. D.C. Hoffman* and H. Donovan, Behavioral Biology, Neurogen Corp., Branford CT 06405.

The noncompetitive NMDA receptor antagonist MK-801 prevents the development of sensitization to the locomotor-activating effects of amphetamine (Karlof et al., 1992; Wolf and Khanna, 1991). In the present study, the possibility that the NMDA receptor might also play a role in the rewarding effects of amphetamine (as measured in the conditioned place preference paradigm) was investigated. Male Sprague-Dawley rats received amphetamine (2.0 mg/kg IP) paired with one side of a two-compartment box and saline paired with the other side. During these pairings, locomotor activity was measured. On the test day, the amount of time drug-free rats spent in each compartment was determined. Rats treated with amphetamine alone showed no change in time spent in the salted-compartment versus the saline-compartment. Rats treated with MK-801 alone showed no consistent place conditioning effects, although a place preference was observed at the intermediate dose. On conditioning days, MK-801 produced a dose-dependent enhancement of amphetamine-induced locomotor activity, however, MK-801 alone caused a significant increase in activity. These data suggest that the NMDA receptor is probably not involved in either the rewarding or locomotor-activating effects of amphetamine.

**657.3**

DZ-063 (MK-801) POTENTIATES LOW-DOSE FACILITATION OF BRAIN STIMULATION REWARD BY BOTH AMPHETAMINE AND MORPHINE. W.A. Carton*, R.S. and R.A. Hug, Center for Studies in Behavioral Neurobiology, Concordia Univ., Montreal, Quebec, Canada H3G 1M8

It has been reported that the non-competitive NMDA antagonist dizocline (MK-801) can block both the behavioral sensitization observed after repeated amphetamine and the tolerance and dependence observed after repeated morphine. The purpose of the present study was to evaluate the effects of blockade of the NMDA receptor on the reward-facilitating effects of amphetamine and morphine using brain stimulation reward (BSR). In rats (n=8) with stimulating electrodes aimed at the medial forebrain bundle (MFB), acute administration of a low dose of amphetamine (0.25 mg/kg, IP) caused a leftward shift in the function that relates stimulation frequency to response rate, causing a 14% decrease in BSR threshold. Dizocline, at a dose of 0.05 mg/kg (IP) that elicited a minimal (9%) decrease in threshold, potentiated the threshold-lowering effects of amphetamine: the combination produced a 29% decrease in threshold. Furthermore, after tolerance had developed to its sedative side effects, a small (12%) decrease in threshold could be observed after administration of morphine (2.5 mg/kg, IP); this threshold-lowering effect was also potentiated (to 23%) by dizocline. These effects do not appear to be due to sensitization to amphetamine, morphine, or dizocline. Thus, the present results extend the finding of the noncompetitive NMDA antagonist, MK-801, which is coadministered with the noncompetitive NMDA antagonist MK-801. The nonselective DA antagonist haloperidol partially attenuated this effect. These data suggest that the NMDA receptor is probably not involved in either the rewarding or locomotor-activating effects of amphetamine.

**657.4**

AMPHETAMINE SENSITIZATION AND NMDA RECEPTORS: ALTERATIONS OF THE Dopamine (DA) AND NMDA RECEPTORS IN AMPH SENSITIZATION. Rats were treated with either saline or MK-801 (0.25 mg/kg, i.p.) for 5 days and challenged with MK-801 after 3 days off. Rats treated with either saline or MK-801 (0.25 mg/kg, IP) were injected with either saline or MK-801 (0.25 mg/kg, IP) 30 min later by AMPH. The following were performed after 3 or 10 days off: 1) electrophysiological studies of postsynaptic D1 and D2 receptor sensitivity in the nucleus accumbens (NAc), 2) electrophysiological studies of DA autorceptor sensitivity in the ventral tegmental area (VTA), and 3) microdialysis studies of AMPH-stimulated DA levels in the NAc. AMPH-treated rats were behaviorally sensitized at both 3 and 10 days off. At 3 days off, autorceptor sensitivity in the VTA and AMPH-stimulated DA release in the NAc were similar in the saline and AMPH groups. However, supersensitivity of both D1 and D2 receptors in the NAc was observed in the AMPH group. MK-801 coadministration prevented the development of D1 receptor supersensitivity. Studies at 10 days off are in progress. Supported by DA-07755 (MEW), DA-04083 (FWJ), DA-06893 (FWJ), NARSAD (MEW), the PMA Foundation (MEW) and Institutional NRSA GM-08164 (FWJ).

**657.5**

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) INHIBITS GLUTAMATE-EVOKED FIRING OF NUCLEUS ACCUMBENS CELLS. S.R. White* and K.C. Paros, Dept. of VCAPP, Washington State Univ., Pullman, WA 99164.

MDMA is a recreationally used amphetamine derivative that has been reported to release serotonin (5HT) and, less potently, dopamine (DA) from nerve terminals in the forebrain (Schmidt et al., Biochem. Pharmacol. 26, 1977, 747). The nucleus accumbens (NAc), a major component of the brain reward pathway, is innervated by both 5HT- and DA-containing nerve terminals. This study examined the effects of local application of MDMA on excitability of NAc neurons in vitro. Extracellular single unit recording was combined with microiontophoretic drug application in urethane-anesthetized male rats. NAc cells were driven at a slow, stable firing rate by cycled pulses of glutamate. Microiontophoretic application of MDMA (10-40 nA, 60 sec) produced a slowly developing, dose-dependent inhibition of glutamate-evoked firing that was not blocked by administration of equivalent currents applied to a pH control solution. Application of 5HT and of DA produced a dose-dependent inhibition of glutamate-evoked firing that was similar to the effect of MDMA. The nonselective DA antagonist haloperidol partially attenuated this effect. These data suggest that MDMA-induced inhibition of NAc neuronal excitability may be mediated by both DA and 5HT receptors.

**657.6**

THE NONCOMPETITIVE NMDA ANTAGONIST, MK801, BLOCKS THE DEVELOPMENT OF SENSITIZATION TO THE LOCOMOTOR ACTIVITY EFFECTS OF AMPHETAMINE. I. Stewart*, M. Akar, J.L. Moore. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada H3G 1M8.

Several studies have shown that the NMDA antagonist, MK801, blocks the development of sensitization to amphetamine-induced activity. Here we show that this effect cannot be explained by interference by MK801 with the release of dopamine (DA) by amphetamine in that MK801 also blocks the development of sensitization to the direct mixed DA receptor agonist apomorphine (APO). During preexposure, rats were pretreated with either 0.25 mg/kg MK801, 1.p., or saline prior to injections of 2.0 mg/kg APO HC1, i.p. or saline, on five occasions every three days. Group PAIRED received APO in the activity boxes and saline in the home-cage, group UNPAIRED, saline in the activity boxes and APO in the home-cage, and group CONTROL, saline in both places. All animals were tested for conditioned place preference following intraperitoneal injection of saline and for sensitization following 0.5 mg/kg APO.

**657.7**

AMPHETAMINE SENSITIZATION AND NMDA RECEPTORS: NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL CORRELATES IN THE MesoACCUMBS DOPAMINE SYSTEM. M.E. White, F. Wolf, F.A. White, R.L. Broderson and M.R. Khanna. Departments of Psychiatry and Pharmacology, Wayne State University School of Medicine, Detroit, MI 48207.

We have shown previously that administration of MK-801, a noncompetitive N-methyl-D-aspartate (NMDA) antagonist, results in sensitization to the locomotor stimulant effects (Brain Res. 565: 164, 1991). The present study sought to further characterize this phenomenon. Rats were treated with either saline or MK-801 (0.25 mg/kg, IP) for 5 days and challenged with MK-801 after 3 days off. Studies in which the D1 antagonist SCH 23390 (50 mg/kg, IP, 15 min before MK-801) was coadministered on either the treatment days or the test day indicated that neither the development nor expression of sensitization to MK-801 required dopamine (DA) receptor stimulation. Microdialysis studies demonstrated that MK-801 challenge was associated with a modest (10-15%) increase in DA levels in the nucleus accumbens and that this effect was not augmented in MK-801 sensitized rats. Electrophysiological studies showed that repeated administration of MK-801 resulted in supersensitivity of both D1 and D2 receptors in the nucleus accumbens. These results suggest that MK-801 sensitization does not require DA receptor stimulation for its induction but may share other features with sensitization to amphetamine and cocaine. Supported by DA-07755 (MEW), DA-04083 (FWJ), DA-06893 (FWJ), NARSAD (MEW), the PMA Foundation (MEW) and Institutional NRSA GM-08164 (FWJ).
657.13

AMPKAINAX RECEPTORS IN THE NUCLEUS ACCUMBENS ARE INVOLVED IN MEDIATING CONDITIONED PLACE PREFERENCE ELICITED BY AMPHETAMINE, COCAINE, AND MORPHINE. F.G. Kadish, R.T. Layer, N.J. Utley*, and J.L. Wallace. College of Pharmacy, Ohio State University, Columbus, OH 43210.

Activation of AMP/kinase glutamatergic receptors in the nucleus accumbens (NACC) may be a component of the mechanism of drug induced reward. To test this, the antagonist DNQX was injected into the NACC just prior to administration of amphetamine, cocaine, or morphine during the training phase (acquisition) of a conditioned place preference ( CPP) paradigm. Rats were then tested for CPP in the absence of drugs. In other experiments, DNQX was given just prior to testing for place preference (expression) but not during training. Bilateral intra-NACC administration of DNQX (1 µg/0.5 µl/probe) inhibited acquisition of CPP induced by amphetamine (1 mg/kg) and cocaine (20 mg/kg) but not morphine (10 mg/kg). During acquisition, DNQX attenuated the locomotor stimulation elicited by amphetamine during the first but not subsequent sessions and that by cocaine during all training sessions. However, DNQX made morphine treated rats akinetic. When given prior to testing, DNQX inhibited the expression of CPP induced by amphetamine and morphine (experiments with cocaine are in progress) but did not affect locomotor activity. Our results suggest that activation of AMP/kinase receptors is involved in the primary reward stimulation (acquisition of CPP) of psychostimulants but not opiates and in behaviors related to memory of primary reward stimulation (expression of CPP) for both classes of drugs. Furthermore, locomotor activity during conditioning is not necessary for acquisition of CPP.

DEGENERATIVE DISEASE: PARKINSON'S V

658.1


Monoamine oxidase (MAO) is the enzyme responsible for the neuronal metabolism of aminergic transmitters and exists in two isoforms, MAO-A and B. We have previously shown that daily administration of deprenil (DEP), an irreversible MAO-B inhibitor, to rats and mice at doses acutely selective for inhibiting MAO-B results in cross-inhibition of MAO-A in the CNS (Heikkila, 1990). This effect is both time and dose-dependent and has also been observed in Parkinsonian patients who received DEP for 1 week (Riederer, 1988). To further examine the effects of chronic DEP administration, male Sprague-Dawley rats were given either 0.2mg/kg or 0.02mg/kg DEP sc for 10 consecutive days. Another group of animals received a single injection of 0.2mg/kg DEP. Animals were sacrificed 4 days following the final inhibitor administration to allow for 20-30% recovery in enzyme activity. CI"l-Benzylamylne was used as substrate for MAO-B in a radioenzymatic assay to measure K m and V max in neostriatum (STR) and remaining cerebral hemispheres (CER). In both regions, V max was similar between the 0.2 and 0.7mg/kg groups (STR = 1.72nmol/mg tissue/hr, CER = 2.11) which differed from the 0.02mg/kg group (STR = 2.12, CER = 2.83). In STR, MAO-A inhibition occurred only in the 0.2 mg/kg group (13%) when serotonin was used at V max conditions. A significant decrease in K m occurred in STR of animals receiving 0.02mg/kg (15.3±10.8) compared to naives (97±22), whereas the 0.2mg/kg and 0.7mg/kg groups displayed insignificant changes. However, no alteration in K m was observed in CER. Whether the difference in K m reflects an allosteric modification of MAO-B or expression of a different isoform remains to be elucidated.

658.2

CYCLIC AMP, BUT NOT BASIC FGF, INCREASES THE IN VITRO SURVIVAL OF MESENCEPHALIC DOPAMINERGIC NEURONS AND PROTECTS THEM FROM 6-HYDROXYDOPAMINE INDUCED DESTRUCTION. J. Hartlicka*, and H. Lohberger. Preclinical Research, Sandox Pharma Ltd., CH - 4002 Basel, Switzerland.

We have identified substances which affect the survival of dopaminergic neurons using primary cell cultures prepared from fetal (E14) rat substantia nigra. Exposure of mesencephalic cultures to forskolin or cAMP analogues during the first three days after plating increased the dopamine uptake activity by 100-200%. The response of dopaminergic neurons to forskolin could be greatly enhanced by exposing the cultures simultaneously to phosphodiesterase inhibitors. IRX or Rv 20-174. In 3-day old cultures treated with forskolin or cAMP analogues, the number of dopaminergic neurons was increased by 50%. Exposure of cultures to 1 µM of MPP+ for 24 hours at the end of a 6-day long culture period reduced the number of TH+ neurons by 50%. Cyclic AMP, but not basic FGF, was able to prevent the degeneration of dopaminergic neurons induced by MPP+. The results suggest that increased intracellular levels of cAMP protect dopaminergic neurons in situations of stress like the process of dissociation, or the exposure to neurotoxic compounds. Our results reveal novel possibilities for the treatment of Parkinson's disease.

658.3


Treatment of Parkinson's disease by transplantation of fetal brain tissue may be complicated by the effects of the continued administration of L-DOPA on the development (DA) neurons in the graft. Cell cultures obtained from mesencephalic brain tissue of rat embryos 13 to 21 days of gestation were used to study the toxic effects of L-DOPA on the development, survival and function of DA neurons. DA neurons were identified and characterized using neurochemical (HPLC analysis of monoamines, uptake of exogenous tritiated dopamine and activity of tyrosine hydroxylase) and immunohistochemical techniques. DA neurons show extensive fiber formation after visualization using an antibody to tyrosine hydroxylase. DA and 3,4-Dihydroxyphenylacetic acid levels in the cultures reach a plateau after 10 days in vitro. Cocaine-sensitive, tritiated DA uptake increased to a plateau between 3 and 7 days in vitro whereas DA uptake increased for up to 17 days. Addition of 5x10-5 or 10-4M DHX to the culture media for 4 days resulted in cellular degeneration, a 50-55% decrease in DA levels and a total loss of cocaine-sensitive tritiated DA uptake. Concentrations of 10-5 and 10-4M DHX decreased 20-30% decreases in DA levels and 10-30% decreases in cocaine-sensitive tritiated DA uptake. The demonstration of morphological and biochemical changes in cultured DA neurons after short-term administration of DHX suggests that L-DOPA may affect the development of transplanted fetal DA neurons. Supported by the G. Harold and Leila Y. Massey Charitable Foundation, the United Parkinson Foundation and the National Parkinson Foundation.

Although post-synaptic compensatory changes following denervation with 6-hydroxydopamine, few studies have evaluated such effects after MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) treatment. C57BL/6 mice were used to evaluate possible functional supersensitivity of striatal D1 receptors, since MPTP produces selective degeneration of substantia nigra-caudate neurons in this strain. Olfactory bulbectomy was used as a probe because it is a point-of-no-return model of denervation and lacks mesencephalic damage.

Results obtained 7 months later will be presented. A single SC injection of 15 mg/kg MPTP. Three weeks after dosing, mice were tested for behavioral supersensitivity, and striatal tissue was collected to measure dopamine concentrations, as well as D1 and D2 effects on regulation of adenylate cyclase. MPTP caused a 75% decrease in dopamine, but did not cause behavioral supersensitivity. D1 receptor activity (BD2, 450-550 nM) was measured in striatal slices. The result of D1-adenyl cyclase activity indicated a decrease in the expression of the enzyme activity in MPTP-treated mice. The decrease was more pronounced in the lateral region compared to the medial area. Parallel studies found that the expression of TH was significantly lower in the lateral compared to the medial region of the substantia nigra. 

658.6 CHRONIC SINEMET (L-DOPA) ALTERS DOPAMINE RECEPTORS IN MPTP-TREATED MONKEYS. L. Rojas*, P.A. Frota, J.S. Schneider and J.N. Joyce. Dept. Psychiatry, Univ. Penn. Sch. Med., and Dept Neurology, Haberman Hospital, PA, USA.

Administration of MPTP to monkeys results in a parkinsonian syndrome that can be reversed by dopamine administration. However, chronic administration of L-DOPA also lead to dyskineticities in parkinsonian animals. In order to understand the involvement of the DA system in this syndrome, the present study examined the regional integration of the DA system using autoradiography for the pre-synaptic DA system (3H][mazindol binding to DA uptake sites), D1 receptors (3H][SCH]23390), and D2 (125I][iodoplatid) in the basal ganglia (caudate putamen, NAc, GPi). Nuclear accumulation (NAs), global, internal (GN) and external (GPe) were analyzed in the anterior striatum of non-dyskinetic compared to dyskinetic animals. The monkeys were divided into three groups: control, MPTP-treated receiving L-DOPA.

The MPTP-treated monkeys were studied 6-11 weeks after the last injection of MPTP and the L-DOPA-treated monkeys 30-48 hrs after the last dose of L-DOPA. In one hemisphere, DA levels were measured for behavioral supersensitivity, and the other for autoradiographic studies. MPTP induced loss of [3H][mazindol binding (> 90%), slightly more in dorsal areas (8%) than ventral ones (9%), while relatively sparing the NAs (64% loss). D2 receptor density and distribution was largely unchanged by MPTP and L-DOPA treatment. In MPTP monkeys, a substantial increase of D1 receptor density was observed in both D1 and D2 receptors in both hemispheres. In the other hemisphere of MPTP monkeys, a further increase of D1 receptor density was observed in regions of striatum, but not in GPi. Interestingly, L-DOPA induced an increase in D1 receptors in the OPs. Contrary to previous reports, D1 and D2 receptor density was increased following MPTP and L-DOPA. L-DOPA-induced D1 increases may be related to an increase in dopaminergic activity. 

Funded by R29 MH 4385, FRSG fellowship, and the Benjia Essential Blphosphorasm Research foundation
In the weaver mutant mouse, a subpopulation of substantia nigra cells is lost during early postnatal development as a result. The development of dopaminergic neurons is greatly decreased and a number of striatal neuronal characteristics are affected. In other models, loss of striatal dopamine causes endogenous opioid peptide and receptors. In rats, neurotoxic lesions of the nigrostrialectomy have been shown to decrease striatal opioid receptors, to decrease striatal dynorphin expression and to increase striatal enkephalin expression. In the weaver mouse, we have recently shown that striatal delta and kappa opioid receptors are decreased as well as controls, while mu receptors are unchanged. In the present study, we compared tyrosine hydroxylase, proenkephalin and dynorphin mRNAs in weaver and control littermate mice using in situ hybridization and quantitative autoradiography. Tyrosine hydroxylase mRNA decreased in the substantia nigra pars compacta of weaver mice, but appeared unchanged in the olfactory bulb. While striatal proenkephalin mRNA did not differ between control and weaver brains, proenkephalin mRNA significantly increased in the weaver striatum (p = 0.005). Thus, enkephalin is upregulated following genetically determined, early postnatal loss of striatal dopamine. The results suggest that upregulation of enkephalin is not necessarily associated with mu opioid receptor downregulation. Parallels between opioid system changes in weaver brains and Parkinson's disease suggest new treatment strategies for symptoms not controlled by L-DOPA, such as dyskinesia or affective disorder. Supported by NS 26761 and the American Parkinson Disease Association SCC.

MPTP does not mimic the effects of Parkinson's disease on the adrenergial medulla of the mouse. S.L. Stoddard*, G.J. Merkx, J.A. Cook, and S.W. Carmichael. Dept. of Anatomy and Microbiology, Indiana Univ. School of Medicine, Fort Wayne, IN 46802 and Dept. of Anatomy, Mayo Clinic, Rochester, MN 55905

We have previously reported that the catecholamine content of the adrenal medulla is depressed in the parkinsonian patient [Stoddard et al. Exp. Neurol. 104, 218-222, 1990]. This condition may be a peripheral manifestation of Parkinson's disease. Since MPTP destroys dopaminergic cells in the substantia nigra and is used to produce a model of Parkinson's disease, we wished to determine whether this neurotoxin also affects peripheral targets. Mice (CS7BL, 6-8 wk-old, N=30) were injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg, 3 mg/mg/ml) at 4, 8, and 24 hours, and sacrificed 4, 8, 12, or 16 weeks later. Catecholamines were extracted from both the adrenal glands and striatum by alumina adsorption, quantitated by high performance liquid chromatography with electrochemical detection, and expressed as ng/adrenal pair or ng/mg striatum. Catecholamine levels in MPTP-treated adrenals were compared to levels in age-matched control adrenals (N=21) using a simple randomized two-factor ANOVA. Although a slight decrease in catecholamine content was observed 4 weeks in the MPTP-treated animals, the difference was not significant and catecholamine levels returned to control levels over time. These data suggest that, although MPTP mimics the central effects of Parkinson's disease by decreasing the dopamine content of the striatum, this neurotoxin does not produce the concomitant depletion of adrenal medullary catecholamines that is observed in human patients with idiopathic Parkinson's disease.

The biochemical effects of 5-adenosylmethionine (SAM) in rats: relationship to Parkinson's disease. B. Crowell, Jr.* and C. Charlton. Dept. of Physiology, Meharry Medical College, Nashville, TN 37212

S-adenosylmethionine (SAM) is a potent methyl donor that is involved in the metabolism of several biogenic amine neurotransmitters, including dopamine (DA), norepinephrine (NE) and serotonin (5-HT). When SAM is injected into the lateral ventricle of rats, tremors, hypokinesia, and abnormal posture are observed. These impairments along with the depletion of DA, NE and 5-HT have been observed in Parkinson's Disease (PD) patients. The SAM may be involved in PD. The mechanism by which SAM induces motor impairments in rats is believed to be related to a depletion of DA.

We tested these hypotheses by injecting SAM into the lateral ventricle of rats and measuring changes in DA, NE and 5-HT. Measurements were made acutely (one injection and determination at 20 post-injection) and chronically (daily injection for 14 days and determination at 16 days post-injection). Both the acute and chronic administration of SAM depleted DA by 26.6 and 41.8% and increased HVA/DA ratio by 42.3 and 47.9% respectively. NE was depleted by 30.3% following the acute administration of SAM, whereas, 5-HT was depleted by 60.1% following only the chronic administration. These results showed that the biochemistry of DA, NE and 5-HT are affected following the administration of SAM. Therefore, the administration of SAM into the lateral ventricle of animals may serve as a useful tool to study the effects of SAM and the already established behavioral effects indicate that excessive SAM-dependent reactions may be involved in PD. Supported by NIH GM08037 and NS 29432

The effect of estrogen upon L-DOPA evoked striatal dopamine release in vitro in MPTP-treated female mice. J.L. McDermott* and D.E. Hughes, Department of Geriatric Medicine, University Hospitals, Cleveland, OH 44105, and Department of Anesthesiology, Northeast Ohio Universities College of Medicine, Rootstown, OH 44272.

Ovariectomized (OVX) rats, ovariectomized CD-1 and C57/Bl mice ± estrogen (EB) were treated with MPTP (10 mg/kg, i.p.) at 24 h intervals for 10 days (n = 10). At 5-10 days post-MPTP, L-DOPA (5 m M) evoked dopamine (DA) release from superfused corpus striatal tissue fragments was determined. Estrogen treatment of CD-1 mice increased L-DOPA-activated DA release (6 ± SEM - areas under L-DOPA-evoked DA release curves in pmoles/10 min: OVX/MPTP = 316 ± 53, N=12 versus OVX + EB/MPTP = 494 ± 104, N=11). Moreover, MPTP/DAMGO-(6-8) antagonists that were obtained in estrogen-treated CD-1 mice (OVX + EB = 511 ± 48, N=12) in contrast, estrogen treatment of C57/Bl mice treated with MPTP did not significantly alter L-DOPA-evoked DA release (OVX/MPTP = 372 ± 89, N=10 versus OVX + EB/MPTP = 303 ± 37). However, MPTP did significantly reduce L-DOPA-evoked DA release in these C57/Bl mice (OVX + EB = 579 ± 69, N=11). In both strains estrogen treatment resulted in significant increases of L-DOPA-evoked DA release (CD-1: OVX = 335 ± 56, N=12 versus OVX + EB = 511 ± 48, N=12; C57/Bl: OVX = 219 ± 46, N=12 versus OVX + EB = 579 ± 69, N=11). These results demonstrate that DA mice of both strains show enhanced striatal DA responsiveness to L-DOPA following estrogen treatment. Interestingly, while MPTP treatment did not reduce L-DOPA-evoked DA release, estrogen treatment of female mice did. Estrogen dependent augmented L-DOPA evoked response was completely abolished in C57/Bl, but not CD-1, mice treated with MPTP.

L-DOPA effects on methionine adenosyltransferase (MAT) and catechol-O-methyltransferase: relevance to Parkinson's disease (PD) therapy. R. Benson+, C. Charlton, Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

It was observed that the injection of S-adenosyl methionine (SAM) into the lateral ventricles of rodents produced similar symptoms to those seen in PD patients, namely, tremors, hypokinesia, depletion of tyrosine hydroxylase and dopamine (DA), an increase in homovanillic acid to DA ratio and degenerative changes in the nigrostrialectomy; therefore, increased biological methylation may occur in PD. L-dopa, the major therapeutic agent for PD, is a potent methione acceptor. It utilizes SAM when it is administered. Such a utilization, along with an increase in DA, may be related to the effect of L-DOPA. It follows that the lack of efficacy from prolonged L-dopa therapy may be due to a) a decreased DA receptor, b) increased metabolism of L-DOPA in the same brain region, which tends to replenish the methylation system. To test this, mice were exposed to chronic L-dopa followed by analysis of brain and plasma MAT and COMT activities. L-dopa (100 mg/kg) treatments of 1, 2 and 3 times/day for 4 days increased brain MAT activity by 0.2%, 0.4% (not significant) and 21.3%, respectively. A treatment of 3 times/day for 8 days further increased (28.4%) brain MAT activity. MAT was not detected in the plasma. Preliminary data showed that L-dopa treatments of 1 and 3 times/day for 4 days increased brain COMT activity by 27.9% and 39.1%, respectively, above that of the controls. In contrast, the plasma COMT activity was decreased by over 80%. These results indicate that high frequency and chronic l-dopa treatments will induce increases in the activities of brain MAT and COMT, while decreasing plasma COMT. Increased MAT will increase SAM, which in combination with the increased COMT will lead to the increased metabolism of L-dopa and DA. (Supported by NIH GM09307 and NS 29432)


The potency and intrinsic activity of a dopamine agonist, U-91536A (U), has been compared with drugs apomorphine (A), bromocriptine (B), lisuride (L), quinpirole (Q) and pergolide (P) by measuring the striatal acetylcholine (ACH) concentration in non-reserpinized (NR) and reserpinized (R) rats. Some of the drugs have also been investigated in unilateral substantia nigra (SN) lesioned (6-hydroxydopamine) rats. In NR rats, the ED50s of Q, L, U, P, A and B, in descending order of potency, were 0.06, 0.21, 1.62, 1.72 and 19.0 mg/kg. L failed to increase ACH levels by 50%. U was the most efficacious (100%) compound, and the relative intrinsic activities of Q, T, A and B were 80%, 63%, 45% and 30%, respectively. Reserpine (5 mg/kg) significantly (p<0.01) decreased ACH concentration. In R rats the, ED50s of AP drugs were several fold lower than in NR rats. In R rats, the slope of the dose-response curves for P and L were significantly different from those of U and Q. This may be due to the D2 selectivity of U and Q and non-selectivity of P and L for dopamine receptors. In addition, serotonergic and adrenergic effects of P and L may alter their dopaminergic response. The responses of Q and L for increasing ACH concentrations were significantly higher on the lesion side as compared to the intact side due to supersensitivity of D2 receptors following the degeneration of SN. The effects of AP drugs on striatal ACh concentrations indicate that U-91536A is a post-synaptic dopamine receptor agonist and may be useful for treatment of Parkinson's disease.
DECREASED EXTRACELLULAR BUFFERING CAPACITY DIMINISHES HIPPOCAMPAL SLICE SURVIVAL OF ANOXIA

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The capacity of brain tissue to survive anoxia or ischemia may depend upon how well brain tissue buffers pH changes during and after such insults. This dependency was examined in hippocampal slices from male Sprague-Dawley rats. Slices were transferred into an interface chamber and bathed in an artificial cerebrospinal fluid (ACSF) of control (0.6 mM) or low (5 mM) bicarbonate buffering capacity. Slices were bubbled initially with gas mixtures containing 95% O2, 5% CO2 (control), or 99% O2, 1% CO2 (low NaHCO3). ACSF pH was 7.3-7.4. Slices were made anoxic (95% O2, 5% CO2) (control) or 99% N2, 1% CO2 (low NaHCO3) for approximately 9 min., then returned to the appropriate oxygen-containing gas mixture. During an experiment, extracellular K+ activity (K+) and extracellular Na+ activity (Na+) were monitored. During anoxia, disappearance of the orthodromic population spike occurred sooner in low NaHCO3 ACSF. After anoxia, slices exposed to low NaHCO3 were less able to reestablish K+ homeostasis and did not recover population spike activity. These results support the view that sufficient HCO3- buffering capacity is required for recovery of ion homeostasis and synaptic transmission in brain tissue following anoxia. (Supported by NIA AG08710)

SNARF AND NEUTRAL RED INDICATE THE PRESENCE OF DISTINCT HYDROGEN ION COMPARTMENTS IN THE RAT HIPPOCAMPAL SLICE.


We have previously reported the effects of amiloride analogs on intracellular pH estimated by the Neutral Red spectrophotometric method. We have extended these studies, comparing the pH estimates made from SNARF fluorescence to those of Neutral Red. The difference in control values and in response to anoxia or ammonium-ionophoresis to demonstrate that these methods are monitoring separate pH compartments. Hippocampal slices were exposed to 50 µM Neutral Red or 10 µM SNARF-AM in HEPES-buffered artificial cerebrospinal fluid (ACSF) for 1 h prior to a 20 min anoxia period to remove extracellular dye. The slices were transferred to an optical recording chamber and monitored for 30 min in control conditions. The ACSF was then switched to one of three experimental paradigms: 1) ACSF equilibrated with 100% nitrogen to produce anoxia; or 2) ACSF containing 10 mM amiloride to block sodium-hydrogen antiport; or 3) ACSF containing 20 mM ammonium chloride. The control pH value for SNARF was 7.05 ± 0.01, compared to 7.39 ± 0.05 for Neutral Red. In response to anoxia, SNARF pH dropped to 7.25 units in 10 minutes, and reached a value of 6.75 following 30 min of anoxia. In contrast, Neutral Red pH changed little in the first 10 minutes and was 7.08 after 30 min. Amiloride had no significant effect on SNARF pH during a 60 min exposure, but Neutral Red pH fell 0.8 units to 6.76. Finally, rebound acidification following exposure to 20 mM ammonium chloride caused SNARF pH to acidify by about 0.2 units with no subsequent recovery. Neutral Red pH also fell about 0.3 units, but recovered to control pH level in 10-15 minutes. Initial photographs indicate that SNARF appears to be more highly concentrated in the vicinity of the hippocampal neuronal layers, while Neutral Red exhibits a more uniform distribution in the slice.

SNARF appears to be more highly concentrated in the vicinity of the hippocampal neuronal layers, while Neutral Red exhibits a more uniform distribution in the slice. The metabolic state of the BZ was determined at one hour of reflow following increasing periods of focal ischemia. The MCA was occluded for 1, 2 or 4 h after which perfusion was initiated and the brains were frozen in situ at 1 h of reflow. The brains were sectioned, lyophilized and ATP, P-creatine, glucose, lactate, GABA and glutamate were measured in the cerebral cortex adjacent to the ischemic core. While glucose recovered in the BZ of all groups, lactate levels were 2-.4-5 and 11.8-fold over those of control and ATP levels were 61, 42 and 22 % of control at 1 h of reflow after 1, 2 and 4 h of reflow, respectively. Accumulating these metabolic changes were significant increases in GABA and decreases in glutamate. GABA levels were 1.6-.3-6- and 7.5-fold greater than those of control, while those for glutamate were 76, 64 and 36% of control at 1 h of reflow after 1, 2 and 4 h of ischemia, respectively. These metabolic derangements in the BZ during reflow following increasing periods of focal ischemia indicate that a persisting lesion in the metabolic machinery during reperfusion may be a factor in the evolution of border zone damage following focal ischemia.
HYPOXIC SYNAPTIC DEPRESSION IS A RESULT OF ALTERATIONS AT A PRE-SYNAPTIC SITE AS REVEALED BY THE STUDY OF MINIATURE EXCITATORY POST SYNAPTIC CURRENTS


It is well established that hypoxia will result in suppression of neuronal activity by alterations at the synapse. It is less clear whether this represents a pre- or post-synaptic effect. We examined this by evaluating changes in miniature excitatory post-synaptic currents (mEPSCs) as well as the response to pressure-ejected glutamate following hypoxia in in vitro hippocampal slices using whole cell patch clamp. Glutamate pressure ejector (WPE) studies were performed in the presence of tetrodotoxin (TTX) to block synaptic activity. The amplitude of mEPSCs was evaluated at times during which there was nearly 100% blockade of the orthodromically-evoked excitatory postsynaptic potentials (EPSPs) and suggests a dominant role of pre-synaptic factors in determining anoxic synaptic failure.

(Supported by the Medical Research Council of Canada)
661.1 Acute Outcome Evaluation after Experimental Focal Cerebral Ischemia. Masaaki Uno, M. Christopher Wallace*. Cerebrovascular Research Lab., Univ. of Toronto.

This study was designed to evaluate acute outcome methodology in 3 models of experimental focal cerebral ischemia. Fisher 344 rats were used in 3 different models: (1) proximal middle cerebral artery occlusion (p-MCAO) via craniectomy and MCAO with lateral common carotid occlusion (ipsi-COO) and intravascular MCAO (+1-MCAO) with a 4-6 s occlusion. Sacrifice was performed at time intervals of 1-4 hours after ischemia. Outcome methods included: tetrazolium Chloride perfusion fixation (TTCP) (n=45), Neutral Red (NR) injection (n=20) or 4'-Fluorokinin binding (FK-b) autoradiography (n=25). There was well correlation between infarction volume (TTCP) 83.4±32.1% with p-MCAO at 4 hours. Similar results were obtained with cortical infarction (66.4±23.3, 69.9±19.3) with distal MCAO and ipsi-COO. FK-b in caudate dropped (112±36 vs 187±22pmol/g) at 30 minutes post ischemia. Discrepancy between TTCP (87.3±34.8) and histology (34.5±36.4) was found after 1-MCAO. NR demonstrated no changes in this model at 4 hours. Acute outcome evaluation affords continual monitoring of physiologic variables and can be used in choosing the appropriate model and acute outcome methodology.


The effect of different durations of cerebral ischemia produced by bilateral carotid occlusion for 5, 10, or 15 minutes was determined on performance of a spatial memory test by male Mongrel gerbils. After recovering from the lesion the gerbils were trained in a Morris water maze to swim to a hidden platform which was located in a fixed location. Following 10 daily tests in the Morris water maze the animals were sacrificed and their brains were examined for hippocampal damage. The relationship between the duration of the ischemic insult, the extent of hippocampal lesions and the degree of impairment in the memory task was defined. In a second study, the ability of hyperthermia (30°C) was studied using both histological and behavioral measures. Maintaining low body temperature during ischemia markedly decreased both indices of ischemic damage.


Perinatal asphyxia occurs in 5% of all births and can result in permanent brain damage or death. Neurologic damage is caused by brain hypoxia and ischemia. Despite its clinical importance, few animal models of early hypoxic ischemic brain damage are available. To study this problem we examined the effect of hypoxia and elevated intracranial pressure (ICP) on brain histopathology. We produced hypoxia in isolurane-anesthetized piglets by administering a 1:1 mixture of air and nitrous oxide for 2 h to produce brain ischemia, were saline was injected through a burr hole into the epidural space under the right parietal bone. ICP was maintained at 20 mm Hg above the mean systemic blood pressure for 20 min. We re-expired the ICP and survived the piglets for 2 h. We studied the hippocampal histopathology using a silver impregnation method and screened degenerating neurons. Damaged neurons were found in the CAI/CA4 and hilar regions of the hippocampus. Only the piglets exposed to a combination of hypoxia and elevated ICP died. The piglets exposed to hypoxia alone did not die. The hypoxic ICP piglets should prove useful as a model for determining the mechanisms of hypoxic ischemic brain damage. Supported by a grant from the National Institute of Neurological Disorders and Stroke, and by ECU-SOM Biomedical Research Support Grant 2 S07 RR08812-12.


The intention of this study was to examine the nature of the memory deficits produced by global ischemia in rat. We tested two competing hypotheses that these deficits are produced by glutamate excitotoxicity. Global ischemia was produced in 10 male Wistar rats by bilateral cauterization of the vertebral arteries of the neck. In contrast, in Experiment 2, the unineuromic rats were impaired in a spatial DNNMS task that required them to remember which arm of a Y-maze they had previously entered. Moreover, choice accuracy of the ischemic rats decreased dramatically as the retention interval between forced and choice runs increased from 30 to 120 s, whereas the accuracy of the controls was unaffected. In Experiment 3, injections of the NMDA receptor antagonist, dextromethorphan (20 mg/kg ip), 1 h before and 1 h after the onset of ischemia did not affect the impairment produced by ischemia in the Y-maze. These results indicate that CA1 hippocampal damage produced by global ischemia produces a deficit in working memory, i.e., the capacity to remember where they have been (Experiment 2), but not the capacity to form a spatial map, i.e., to localize where they are (Experiment 1). Moreover, Experiment 3 indicates that this deficit was not caused by glutamate excitotoxicity.

Previous studies in this laboratory have described a decrease in the uptake of 2-deoxyglucose in the cortical barrel field 5 days following trans-frontal section of the sensorimotor cortex. The present study sought to determine the behavioral correlates of this "functional diaschisis" by assessing the effect of a phototochemical injection of the frontal cortex on an appetitively motivated response in a T-Maze using a vibrissa deflection cue.

Adult male Wistar rats were randomly assigned to one of two groups. Rats in the first group were trained to turn right in the T-Maze in response to a right vibrissa stimulation, and left in response to no vibrissa stimulation; the second group were trained to perform the opposite response. Rats received 50 randomly arranged trial trials and 25 left or right trials until the behavioral criterion was achieved (80% or better correct on both the left and right trials).

Following training, focal insults to the left frontal cortex were produced in half of the animals in each training group by the injection of a photosensitive dye (rose bengal) injected into the rats' tail vein, and the direct irradiation of the cranium with a light beam (Watson et al., 1985). The other half of the animals underwent the same procedure, except saline was injected in place of rose bengal (sham). Behavioral testing starting 24 hours after the insult revealed no radical deficits in the intact and right turning of lesioned animals relative to pre-insult levels. These deficits recovered pre-insult levels within 3-4 days following the insult. Sham exhibited no behavioral deficits. These results indicate that frontal cortex infarctions are associated with age-appropriate deficits in the performance of a task that requires cortical barrel field integrity. Supported by NS 05620.

661.8 EFFECTS OF LIPOPOLYSACCHARIDE ON VON WILLEBRAND FACTOR AND FACTOR VIII/PRODUCTION IN RATS WITH AND WITHOUT RISK FACTORS FOR STROKE. D.A. Doore*, E. Heldman, A. Stien*, H. Harvey B. Pollard*, and Jode M. Hallahan. LabCorp, NDID/RM, NINDS, National Institutes of Health Bethesda, Maryland 20892 and "Department of Neurology Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.

We have previously reported that a provocative dose of Lipopolysaccharide (LPS), rats with the risk factor for stroke hypotension developed more stroke events than normal rats. We have also demonstrated that hypotensive rats (SHR) produce more TNFa, in blood and neutrophilic fluid than normotensive rats (WKY) when challenged with LPS injected iv or iv. It is therefore possible that monokines, may act by changing levels of intravascular mediators of thrombosis and cell migration such as was shown for TNFa, on factor VIIIa. To test this hypothesis we asked whether these factors were differentially affected by LPS in SHR and WKY. We report here that hypotensive rats have higher levels of TNFa, than control rats after a single injection of LPS. When SHR rats were injected with a single iv dose of 1.8 mg/kg of LPS, WVF concentration increased in a time and dose dependent manner, reaching a peak 2 hr and with a maximum response at a dose of 1.8 mg/kg. When the response of the hypotensive rats to a challenge with LPS was compared with the normotensive rats, the SHR animals showed significantly higher levels of WVF compared to WKY rats, after a 1.8 mg/kg of LPS was injected either iv or iv. No significant differences were found at lower doses. LPS injected at a concentration of 1.8 mg/kg had an inhibitory effect on factor VIIIa activity which resulted in an increase in the WVF/TFPI ratios. These and other data strongly suggest that the effect of LPS in vivo is not direct on the endothelial cells but most likely mediated through the release of monokines by activated macrophages and microglia.


We investigated the nature of acoustic-induced myoclonus (AIM) in rats that had undergone cardiopulmonary arrest and resuscitation, an insult that is known to produce spontaneous myoclonus and AIM. After 3 days to 4 months AIM was measured using two methods: visual scoring and quantitative measurement of whole-body AIM force. AIM was non-habitualising, regardless of the length of time after the insult, within sessions; the force of the response increased then decreased between sessions run on a single day and decreased slowly over sessions run on sequential days. In addition, AIM was supramaximal, compared to normal controls, shortly after the insult but declined to subnormal several weeks later, regardless of prior testing. The waveform of the force of AIM was significantly different from control animals: there was a decreased latency to the AIM rise and an increase in the initial response. In addition, a secondary longer latency response was present that was not seen in controls. AIM was decreased by the anti-myoclonic drug clonazopam (0.5 - 1.0 mg/kg), GTP (50 - 300 mg/kg), TMPP (0.5 - 2.0 mg/kg), and valproate (100 - 300 mg/kg). These data indicate that cardiac arrest in the rat gives rise to a model of human post-hypoxic myoclonus (PHM) that can be further studied to understand the mechanisms of PHM and its therapeutic treatment.


Recently we have used a SAC to study the effect of high +Gz (head-to-foot) exposure on cerebral metabolism and to understand the mechanism of gravity induced loss of consciousness (G-LOC). G-LOC has been proposed to result from a decrease in cerebral blood flow (CBF) during +Gz exposure. We have shown that a 30 sec +55 Gz exposure causes G-LOC and global ischemia in mice (Soz. Neurosci. Abstr., Vol. 17(2):1991). We propose that G-LOC may occur due to a decrease in energy and minimize acidosis during +Gz exposure. Measurement of CBF in the dynamic environment of SAC is difficult. The brain is heterogeneous in structure, function and CBF. Therefore the use of the sensitive to measure brain regional energy metabolism during +Gz stress. Methods: Fully awake mice were exposed to +55 Gz for 30 sec in the SAC (to induce global ischemia) and brain sampled at various times during and after deceleration by microwave fixation. The brain was dissected in 5 regions and tissue extracts were analyzed for metabolites and total iron to determine the BV. Control mice were exposed to +0.5 Gz for 30 sec and the brain sampled. Results: Data: Deceleration exposure decreased levels of lactate and pyruvate and regional energy metabolism during +Gz stress. Changes were present in all regions after 30 sec of +Gz exposure. Measurement of CBF in the dynamic environment of SAC is difficult. The brain is heterogeneous in structure, function and CBF. Therefore the use of the sensitive to measure brain regional energy metabolism during +Gz stress. Methods: Fully awake mice were exposed to +55 Gz for 30 sec in the SAC (to induce global ischemia) and brain sampled at various times during and after deceleration by microwave fixation. The brain was dissected in 5 regions and tissue extracts were analyzed for metabolites and total iron to determine the BV. Control mice were exposed to +0.5 Gz for 30 sec and the brain sampled. Results: Data: Deceleration exposure decreased levels of lactate and pyruvate and regional energy metabolism during +Gz stress. Changes were present in all regions after 30 sec of +Gz exposure.

Intravenous injections of the fluoroscein rose bengal dye and intense focal light illumination were used to evaluate the development of reproducible models of cerebral and retinal ischamia (Mosinger J. L. and Olney J. W., Exp. Neurol., 103: 172). This procedure resulted in intense fluorescent label of the retina using photochemically-induced neuronal damage to obtain a reliable model to test drugs possibly active against ischemia. Rate were restricted to matched with a pilot study. One eye was exposed to cold light (peak absorption 540 nm) for 1 hr/min. The animals were then sacrificed at different times (1 h and 6 h) and evaluated by light microscopy the retina appeared edema. The microvessels were enhanced and the neuronal showed pyknic vacuoles were also present especially in the inner nuclear and in the ganglion cell layer. The extent of retinal damage was grossly related to the time of illumination. In other experiment the retina used for the determination of GAD (glutamic acid decarboxylase) and CHAT (choline acetyltransferase) activity, two enzymes whose decrease was considered directly related to the extent of retinal damage. The post hoc measurements of GAD and CHAT significantly decreased in a time dependent manner; no changes were present 1 h after the lesion, but 4 h later GAD and CHAT activity decreased by 50% and 40% respectively. The retina illuminated for 15 min indicated a diffuse degeneration of the amacrine cells. This damage remained unchanged for at least 7 days.


Transient forebrain ischemia by the method of four vessel occlusion reproducibly damages the striatum and the hippocampus. However, it has been shown that the substantia nigra melanin (SN) is not damaged acutely by ischemia. In order to test whether delayed injury occurred in the SN animals exposed to 20 minutes of ischemia, the survival period was prolonged, the extent of striatal injury was measured, and the survival rate was determined for non-neural elements. Each of two animals had ischemic damage in the caudate nucleus (CN) and lateral globus pallidus (LGP). The remaining 8 animals had no SN damage, and the ischemic injury was confined to the CN alone. Seven animals were sacrificed 2-3 days after ischemia and none of those had SN damage. Three of these 7 animals had ischemic damage in the CN and LOP, 4 of 7 animals had ischemic injury in the CN alone. All animals demonstrated necrosis or hippocampal injury. The weight data suggest that ischemic injury to CN and LOP are necessary to induce GABA neuron loss in the SN. Animals must survive longer than 3 days for SN injury to become evident. Disinhibition via transneuronal pathways may be one of several possible pathophysiological mechanisms which would account for delayed injury distal from the site of immediate damage.

662.4 EFFECTS OF ANOXIA ON DENTATE GRANULE CELLS. M. Patel, A. DiScenna and D. Kurnan†. Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH - 44106.

Dentate granule cells are considered more resistant to the effects of anoxia, and have not been as extensively studied as CA1 or CA3 neurons (Alden & Schiff, 1986). This work was aimed at investigating the response of granule cells to anoxia and excitation. Intracellular recordings were obtained in vitro, from granule cells of adult rat hippocampus, in control conditions and after exposure to 95% N2, 5% CO2. Anoxic response was observed within 2 min exposure to nitrogen. Extracellular recordings in anoxia showed hypothermia (about 10°C) and a reduction in the amplitude of the population spike, which recovered to 40% of the initial value. Intracellular recordings showed an initial, transient hyperpolarization (3 - 4 mV in 4 of 7 cells examined) followed by a prominent depolarization (50 - 70 mV) in 21 of 23 cells. The onset of depolarization varied between 2-15 min after application of nitrogen, and rates of depolarization were also variable. Dendritic strength-duration data indicated an increase in the orthodromic firing threshold (in 10 cells tested). This effect was reversible (wherever recovery was possible, n = 6). A simultaneous decrease in the EPSP amplitude was measured (in 10 cells: 100% in 4 cells, > 40% in 6 cells). Somatic excitability, as indicated by the response to 100 ms depolarizing current pulses, increased at the onset of depolarization in 6 of 8 cells, but was abolished during further depolarization (in all 4 cells tested). Somatic impedance decreased between 10 - 30% during anoxia. Post-anoxic hyperpolarization was also observed in 4 of 6 cells.

The roles of NMDA receptors and ATP metabolism are being investigated to understand the mechanisms underlying anoxic effects such as the large depolarization observed in the granule cells.

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662.6 SELECTIVE VULNERABILITY OF MOSSY FIBER TARGETS IN THE RAT HIPPOCAMPUS AFTER TRANSIENT FOREBRAIN ISCHEMIA. M. Hux* and G. Burghes. Ctr for Naut. & Behav. Neuroscience, Rutgers University, Newark, NJ 07102.

The vulnerability of different cell types in the rat hippocampus to forebrain ischemia induced by the 4-vessel occlusion method was assessed by immunochemistry using an antibody against the 72kDa heat shock protein (HSP72). Our results showed a robust induction of HSP72-like immunoreactivity (HSP72-Li) in the CA1, CA3 pyramidal neurons. The neuronal somata of CA3 pyramidal neurons were strongly labeled, while CA1 neurons were relatively weakly labeled. The CA1 neurons showed a progressive decrease in labeling intensity as the interval between insult and sacrifice increased. CA1 pyramidal neurons were labeled consistently with antibodies against ChAT, while CA1 and CA3 CA1 and CA3 apical dendrites, restricted to the stratum lucidum of CA3, in the hilus, the horizontal spiny cells were located in the subgranular zone. Both the horizontal spiny cells in CA3 and the hilus were present also at more posterior and ventral locations where no other cell types expressed HSP72-Li. We propose that a determining factor in the susceptibility of these neurons to ischemia is the density of mossy fiber innervation they receive. Furthermore, the effect of ischemia on mossy neuron responses may be related to delayed neuronal death in the CA1 pyramidal cells.

Forbrain ischemia (20 min) was induced in rats by the 4VO method. Ultrastructural observations by CA1 and CA3 were obtained after different survival times (0, 1, 40 and 150 min of reflow) and subjected to quantitative immunocytochemistry using antisera to glutamate, glutamine, GABA, and homocysteic acid. The main observations were: 1) The concentration of glutamate in pyramidal cell bodies and dendrites was substantially reduced during ischemia (probably reflecting a loss to the extracellular space) but in surviving cells it returns to normal after 40 min of reflow. Excitatory-type terminals showed only a minor decrease in the level of glutamate. 2) The glutamate/glutamine ratio in astrocytes was strongly increased at 0 h (reflecting a metabolic block) but returned to pre-ischemic values after 1 h. 3) The level of GABA in interneurons was elevated at 0 and 1 h. 4) A homocysteic acid-like substance appeared in astrocyte processes in CA3 at 48 h and in CA1 at 150 min. The present findings suggest that the latter change is related to the development of delayed neuronal death is currently under investigation.

662.8 EFFECTS OF O2 AND GLUCOSE DEPRIVATION ON HUMAN AND RAT NEOCORTICAL NEURONS IN-VITRO. C. Uang & G.C. Haddad. Section of Respiratory Medicine, Department of Physiology, Yale University School of Medicine, New Haven, CT 06510.

The neocortex is extremely sensitive to O2 and glucose deprivation. Such stress causes rapid loss of corticocortical encephalographic activity and unconsciousness. To study the underlying mechanisms we cultured human and rat cortical neurons in vitro and monitored the loss of neuronal electrical activity during O2 and glucose deprivation, intracellular recordings were performed in the presence of TTX and rats using in-vitro brain slices. Intracellular labelling and electrophysiological characterization showed that most neurons (500-2000 pA) were depolarized immediately after 7 min in rats and 10 min in humans following a transient hyperpolarization (2-5 mV, 1-3 min). The increase of spontaneous EPSPs was suppressed during anoxia, while membrane resistance (300-700 MΩ) remained unaltered with a regular-spiking or a burst firing pattern. Anoxia induced a depolarization of about 20 mV homogeneous after 7 min in rats and 15 min in humans following a transient hyperpolarization (2-5 mV, 1-3 min). Intracellular activity increased by 1-2 µm during anoxia (20 min) or glucose deprivation (30 min), but deprivation of both O2 and glucose elevated K+ by about 25 mM. We conclude that 1) rat neocortical neurons seem to be inherently more resistant to anoxia than brainstem neurons; 2) membrane excitability of anoxic neurons is markedly reduced during anoxia and this is not due only to a decrease in Na+; and 3) anoxic glutamate metabolism in the neocortex is important for neuronal survival during anoxia.

662.9 INVOLVEMENT OF THE NMDA RECEPTOR IN ANOXIA-AGLYCEMIA INDUCED DAMAGE IN THE HIPPOCAMPUS. S. Papas, V. Crepel and J. Ben-Ari. INSERM Unit 29, 123 Blvd. de Port-Royal, Paris, 75014, France.

Although increasing evidence indicates that the NMDA receptor is involved in the induction of anoxic/ischemic injury, its exact role is unclear. For example, protective effects of NMDA antagonists have been found to vary with the ischemic model used. We have therefore used two protocols to examine the involvement of the NMDA receptor in the induction of synaptic transmission induced by anoxia and aglycemia (AA) in CA1 of the hippocampus during anoxia.

In initial studies, the protective effects of a non-competitive NMDA antagonist, MK-801, against AA induced damage was examined. Inhibition and recovery of synaptic transmission was determined by the disappearance and percent recovery of the CA1 field EPSP in the 30 min period after AA insult. Rat hippocampal slices incubated in MK-801 (0.1 µM) showed a 10 min delay prior to 3 min to 4 min 30 s periods (3 min 40 min 30 s of AA (55% 20% 45% of control, p<0.05) and 2 min 30 µM). The exposure of rat hippocampal slices to conditions that are known to reduce energy supplies (hypoxia or glucose deprivation, OD), make them hyperexcitable to the excitotoxic glutamate and N-methyl-D-aspartate (NMDA).

These results indicate that KA receptor channels gate CA1 in addition to OD. Moreover, the results strongly suggest the existence of two distinct KA-receptor subtypes which are differentially activated by hypoxia or GD.

662.10 HYPOXIA OR GLUCOSE DEPRIVATION ACTIVATES TWO SEPARATE KAINATE RECEPTOR SUBTYPES IN HIPPOCAMPAL SLICES. B.M. Rigor*, C.A. West, and A. Schurr. Dept. of Anesthesiology, Univ. of Louisville Sch. of Med., Louisville KY, 40292.

We have tested the effectiveness of the glutamate antagonists in reducing the delayed ischemic damage in the hippocampus. Slices were subject to 2°C 4°C (0 min, 30 min, or 10 min) 10 mM glutamate (Glu) or 10 mM K+O for 15 min prior to various periods (3 min, 30 s to 4 min 30 s) or AA (77 ± 10% in controls, p<0.05). Larger concentrations of MK-801 showed protective effects in more than one time period of AA. In a second investigation, NMCA were enhanced following 3 min 30 s AA (29 ± 13% vs 10 ± 10% in controls, p<0.05). Therefore, we conclude that 1) rat neocortical neurons seem to be inherently more resistant to anoxia than brainstem neurons; 2) membrane excitability of anoxic neurons is markedly reduced during anoxia and this is not due only to a decrease in Na+; and 3) anoxic glutamate metabolism in the neocortex is important for neuronal survival during anoxia.

662.11 QUILNOLATE: A MODULATOR OF GLUTAMATE RECEPTORS? AGONISTIC ACTIVATION IN HIPPOCAMPAL SLICES. A. Schurr*, C.A. West, and A. Schurr. Dept. of Anesthesiology, Univ. of Louisville Sch. of Med., Louisville KY, 40292.

Excitatory amino acids (EAs) in the central nervous system are involved in both in neuronal transmission and excitotoxicity. Quilnolinate (QUIN) is an endogenous tryptophan metabolite and an excitotoxin, and has been shown to play a role in rat brain ischemia. The hypoxic rat hippocampal slice preparation and its electrophysiology were employed to study QUIN’s modulatory role in the activation of the N-methyl-D-aspartate (NMDA) and kainate (KA) subtypes of the glutamate (Glu)-receptor. The degree of neuronal damage in this preparation was used to measure the excitotoxic potential of several GLU ligands. When given at sub-toxic doses, QUIN potentiates the excitotoxicity of GLU, NMDA, and KA in hypoxic hippocampal slices. The MMCA competitive antagonist of the NMDA-receptor glutamic modulatory site, did not. The non-toxic analogue of QUIN, 5-methyl-QUIN, potentiated NMDA toxicity as effectively as QUIN itself. We concluded that QUIN has a specific modulatory binding site on both the NMDA and the KA receptor complex, different from the glycine modulatory site on the NMDA receptor. We propose that QUIN can increase both the excitatory and the excitotoxic efficacy of EAs by potentiating the agonistic activation of GLU receptors.


Organotypic cultures of rat hippocampus could prove useful as an in vitro model of ischemia since the cytoarchitecture and intrinsic synaptic connectivity are preserved. We used hypoxia-hypoglycemia to simulate ischemia and tested the effectiveness of the glutamate antagonists in reducing the delayed ischemic damage in the organotypic culture of the rat hippocampus. The exposure of rat hippocampal slices to conditions that are known to reduce energy supplies (hypoxia or glucose deprivation, OD), make them hyperexcitable to the excitotoxic glutamate and N-methyl-D-aspartate (NMDA).

These results indicate that KA receptor channels gate CA1 in addition to OD. Moreover, the results strongly suggest the existence of two distinct KA-receptor subtypes which are differentially activated by hypoxia or OD.
663.1

Dep. of Neurology, Pharmacology, and Cell Biology and Anatomy, Oregon Health Sciences Univ. and VA Medical Cir., Portland, OR 97201

The Senjii 2-vehicle occlusion model of global forebrain ischemia in rat was used to investigate the time course of changes in specific populations of hippocampal glial cells and neurons following ischemic injury. Astrocytes and microglial cells were immunostained with anti-glial fibrillary acidic protein (GFAP) and anti-cytokeratin, respectively.

GFAP immunoreactivity in astrocytes was reduced in the CA1 and CA3 areas of the hippocampus following brief ischemia. The decrease in GFAP immunoreactivity was most pronounced in the CA1 area, where the reduction was observed as early as 6 hours after the insult. In contrast, the decrease in GFAP immunoreactivity in the CA3 area was observed only at 24 hours after the insult. These results suggest that brief ischemia selectively damages astrocytes in the CA1 area of the hippocampus.

663.2

Neuropathology Lab and Neurology Div. of the Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The development of astrogial and microglial reactions after brief ischemia was studied in models of regional ischemia in baboon and rat. In baboons, ischemia was induced by occlusion of the middle cerebral artery (MCA) followed by reperfusion. GFAP and vimentin were studied as markers of astrocyte and microglial activation.

GFAP immunoreactivity was increased in the CA1 and CA2 areas of the baboon hippocampus following brief ischemia. Vimentin immunoreactivity was also increased in the CA1 and CA2 areas. These results suggest that brief ischemia induces an astrogial and microglial reaction in the baboon hippocampus.

663.3

CLUSTERING OF LIVE AND DEAD ASTROCYTES IN CULTURE: MODULATORY ROLE OF GAP JUNCTIONS. Robert S. Goldstein.
Department of Neurology, Medical College of Wisconsin, Milwaukee, WI 53226.

Iodoacetate (IAA), which interferes with anaerobic metabolism, induces early glial activation in culture in a dose dependent manner. With intermediate doses of IAA, cell death was consistently observed to occur in a distinctly non random spatial distribution; the dead cells formed clusters. Using a two color fluorescence assay and video microscopy, the spatial distribution of dead and live cells was studied over an area of 5x5 mm. Using a modification of nearest neighbor analysis, the extent of clustering was quantified. The clustering effect was robust; the probability of cell death was higher than would be expected based on a random spatial distribution up to hundreds of cells away from a dead cell, and was higher than expected up to hundreds of microns from a dead cell. Although the live and dead cells could represent two subpopulations, the astrocytes stained uniformly GFAP positive and A2B5 negative, suggesting that they represent a single population. Exposure to IAA in the presence of the gap junction blocker octanol (50uM) significantly reduced clustering, though did not entirely eliminate the clustering. This evidence suggests that the non random spatial distribution of cell death is due, in part, to diffusion of a toxic molecule(s) through gap junctions. Previous studies have indicated that IAA-induced astrocyte death is related to elevation of intracellular calcium, raising the possibility that the toxic molecule may be either calcium itself or inositol trisphosphate.
663.5
INVOVLEMENT OF SODIUM CHANNELS IN ANOXIC INJURY IN MAMMALIAN CENTRAL NERVOUS SYSTEM SIMPLE WHITE MATTER: ULTRASTRUCTURAL CORRELATES 
J.A. Black, P.K. Su, B.R. Ransom, and S.G. Wamsy. Dept of Neurology, Yale Univ Sch of Med, New Haven, CT and Neuroscience Research Center, VA Med Ctr, West Haven, CT 06516

The in vitro rat optic nerve has provided a reliable, quantitative model of white matter anoxic injury. Previous studies have correlated optic nerve dysfunction following anoxia with ultrastructural changes in the nerves, which include mitochondrial swelling, cytoskeletal disorganization, and the appearance of empty space adjacent to axons within the nerve. Electrophysiologic evidence suggests that anoxia results in a cascade of events leading to reverse operation of the Na⁺-Ca²⁺ exchanger, and subsequent entry of calcium into the axon. Optic nerves were maintained in Ringers containing 1 mM tetrodotoxin (TTX) during anoxia (60 min). At the end of the anoxic period, the nerves were ultrasonically removed and were maintained in oxygenated, normal Ringers for an equivalent length of time. These experimental nerves exhibited slight swelling of some mitochondria, but neurofilaments, microtubules, and paranodal loops were not apparent in the nerves maintained during anoxia in TTX-Ringers.

Electrophysiologically, these nerves provided morphological evidence for the involvement of sodium channels in axonic injury in white matter and, together with our previous work, suggest the idea that anoxic injury involves sodium influx that leads to reverse operation of the Na⁺-Ca²⁺ exchanger. [Supported in part by VA and NIH]

663.6
EFFECTS OF EXTRACELLULAR CALCIUM ON ULTRASTRUCTURE IN ANOXIC INJURY IN MAMMALIAN CENTRAL WHITE MATTER. 
S.G. Wamsy, J.A. Black, B.R. Ransom and P.K. Su, Dept of Neurology, Yale Univ Sch of Med, New Haven, CT 06516 and Neuroscience Research Center, VA Med Ctr, West Haven, CT 06516

We have developed a reproducible, quantitative model of anoxic white matter injury using the rat optic nerve in vitro. Optic nerves were maintained in Ca²⁺-free Ringers throughout the anoxia period (60 min) and then reoxygenated in normal Ringers. Ultrastructurally, optic nerves in Ca²⁺-free Ringers did not substantially change the ultrastructure of the experimental nerves. These observations provide morphological evidence for the involvement of sodium channels in anoxic injury in white matter and, together with previous work, support the idea that anoxic injury involves sodium influx that leads to reverse operation of the Na⁺-Ca²⁺ exchanger. [Supported in part by VA and NIH]
664.5  
**THERMAL SENSITIVITY OF HYPOXIC RESPONSES IN NEOCORTICAL BRAIN SLICES.** K. Hirama, T. N. Kassell*, K.S. Lee. Dept. of Neurosurgery, University of Virginia, Charlottesville, VA 22908.

Electrophysiological responses to transient hypoxia were studied in neocortical brain slices from gerbils. Evoked responses and DC potentials were recorded in layer III of the frontoparietal cortex under normoxic and hypoxic conditions. The excitatory synaptic component of the evoked wave form was enhanced greatly when transient hypoxia was induced. The delays to the excitatory response were prolonged significantly. Synaptic recovery from a fixed period of hypoxia was enhanced greatly when transient hypoxia was administered at reduced temperature. The data also demonstrate that glutamatergic synaptic transmission is reduced under hypothermic conditions, but that these responses are sustained for a longer period of time during hypoxia.

664.6  

It has recently been shown that hypothermia following ischemia can provide neuroprotection; therefore, the interpretation of the neuroprotective effect of many drugs in confound by drug-induced hypothermia. One technique to eliminate this problem has been to maintain animals in normoxia throughout the post-ischemic period following a hypothermic episode. This study was designed to test the hypothesis that hypothermia after an ischemic episode can provide neuroprotection. The present experiments were conducted to determine whether a shorter duration of temperature maintenance is sufficient to eliminate this confound. Female gerbils were placed on a heated pad and subjected to a 5 minute bilateral carotid occlusion under halothane anesthesia. Immediately after surgery a femoral probe was inserted, the gerbil was restrained in a refrigerated chamber and the probe was connected to a temperature maintenance system (YSI controller, 110 control unit). The temperature was maintained at 36°C for the entire 6 hour period, or at 36.5°C for 2 hours, followed by reduction to 32°C for 4 hours. Brains were harvested 4 days later and hippocampal damage was measured on a 0-2 scale. Six hours of hypothermia in 32°C produced significant neuroprotection, reducing the median brain damage rating to 2, while delaying the onset of hypothermia for two hours produced no neuroprotection (brain damage rating of 3). From these data we conclude that hypothermia begins two hours post occlusion does not provide neuroprotection, allowing for: 1) a shorter period of temperature maintenance when drugs are administered within the first 2 hours after ischemia; and 2) no temperature maintenance requirement for compounds administered after this two hour period.

664.7  

The effect of mild hypothermia on cerebral injury was evaluated in a rat model of permanent (R) middle cerebral artery (MCA) occlusion. The MCA occlusion was performed in rats under halothane at temperatures of 33°C or 36.5°C. Twenty-four hours after MCA occlusion, rats were sacrificed and the brain was extracted, and the percent infarcted volume of the brain was calculated. The data also demonstrate indirectly that hypothermia protects against hypoxic damage to excitatory synaptic mechanisms in the cortex.

664.8  
**INFLUENCE OF THERAPEUTIC HYPOTHERMIA ON THE RELEASE AND UPTAKE OF EXCITATORY AMINO ACIDS.** A.M. Palmer, P.J. Robichaud and J. Copple. Departments of Psychiatry and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Large increases in the extracellular concentrations of excitatory amino acids (EAAs) and a large depletion of the energy charge are considered to play a major role in mediating the secondary brain injury that occurs following cerebral ischemia. Modest decreases in brain temperature (to 30-36°C) during ischemia substantially attenuate neuronal damage in vulnerable brain regions and improve neurologic outcome (J Neurosurg 48: 199-205). In this study, we examined the effects of therapeutic hypothermia on the release and uptake of excitatory amino acids in focal cerebral ischemia. Hypothermic protection was not observed in the ischemic region of the ventrolateral cortex in gerbils subjected to a 90-minute period of focal ischemia. The superfused efflux of preaccumulated D-[3H]glutamate was reduced in animals subjected to hypothermic treatment. However, neither of these changes was influenced by the presence of an excitatory amino acid antagonist. In contrast, hypothermic-induced release of excitatory amino acids was attenuated by another mechanism, probably by diminishing enzyme activity. (Supported by PRP-0182-103).

664.9  
**TEMPERATURE-DEPENDENT CYCLOHEXIMIDE PROTECTION OF HIPPOCAMPAL ISCHEMIC DAMAGE.** G.D. Miller* and J.N. Davis. VA Hosp, Pittsburgh, PA 15213.

To begin to explore factors accounting for hypothermic neuroprotection, we determined markers of nitric oxide production (NOS-2 and cGMP) after 10 minutes of ischemia at 33°C or 36.5°C. We found an increase (ipsi- vs. contralateral MCA cortex) in brain nitrates of 136 ± 102% (n=7) in the 37.5°C group as compared to 19 ± 16 % (n=7) in the 36.5°C group, indicating significant reduction in infarct size by mild hypothermia (P<0.01).

664.10  
**MILD HYPOTHERMIA HAS EFFECTS ON CORTICAL AMINO ACID EFFLUX BUT DOES NOT PROTECT AGAINST PERMANENT FOCAL ISCHEMIA.** E.H. Lo*, R. Newcomb, N. Panahian, N. Mailmeier, G.K. Stromberg. Center for Imaging and Pharmaceutical Research, Harvard Medical School, Boston MA 02115; Neurex Corporation, Palo Alto; and Department of Neurology, University of Stanford, Stanford, CA 94305.

The effect of hypothermia on relationships between the spatial distribution of damage and alterations in amino acid efflux rates in another level of brain injury dependent on the severity of the ischemic insult. In this study, we examined the effects of mild brain hypothermia (33°C, n=7) versus normothermia (37.5°C, n=7) in a rabbit model of permanent focal ischemia (4 hr occlusion of left anterior, middle cerebral, and internal carotic arteries). In vivo microdialysis was used to measure brain extracellular concentrations in anterior ventrolateral and dorsal cortex of the ischemic hemisphere. These locations were defined in previous studies to correspond with regions of dense and boundary zone ischemia respectively. Glutamate was significantly increased by about 1 hr after the onset of ischemia (p<0.05). Glutamine and taurine levels were also reduced. Phospho-ethanolamine was increased about 1 hr after hypothermia. Lowered temperature appeared to increase glutamate efflux in the ventrolateral probe and decreased efflux in the dorsal probe. These results suggest a complex relationship between hypothermia and ischemia. However, neither of these changes was influenced by hypothermia. In contrast, hypothermia-induced increase in the basal efflux of glutamate was attenuated by a dose-dependent fashion. However, neither of these changes was influenced by hypothermia. In contrast, hypothermia-induced increase in the basal efflux of glutamate was attenuated by a dose-dependent fashion. However, neither of these changes was influenced by hypothermia. In contrast, hypothermia-induced increase in the basal efflux of glutamate was attenuated by a dose-dependent fashion. However, neither of these changes was influenced by hypothermia. In contrast, hypothermia-induced increase in the basal efflux of glutamate was attenuated by a dose-dependent fashion. However, neither of these changes was influenced by hypothermia.

Our previous studies indirectly demonstrated that the nerve cell body and terminal responded differently to hypoxia. To directly test this possibility, the responses of cytosolic free calcium ([Ca^{2+}]_i) to K^+ depolarization, hypoxia (RCN), nifedipine and ω-CgTX were compared by fura-2 imaging of the cell bodies and growth cones of NGF-differentiated PC12 cells. Under resting conditions [Ca^{2+}]_i was lower in the growth cones than in the cell bodies. The response to K^+ depolarization was more rapid in the growth cone than in the cell body. Although nifedipine did not alter resting [Ca^{2+}]_i, it impaired the K^+-induced increase of [Ca^{2+}]_i in the growth cone and almost totally blocked the response in the cell body. ω-CgTX nearly abolished the response to K^+ in the growth cone, but did not affect the K^+-induced increase in [Ca^{2+}]_i in the cell body. Hypoxia increased [Ca^{2+}]_i less in the growth cone than in the cell body and neither calcium antagonist altered the hypoxia-induced change. The combination of hypoxia and K^+ depolarization increased [Ca^{2+}]_i more than either alone and the elevation was greater in the cell body. ω-CgTX greatly reduced the exaggerated increase in [Ca^{2+}]_i due to K^+ in hypoxic growth cones, but nifedipine had only minimal effects. ω-CgTX did not alter this response in hyptic cell bodies, but nifedipine greatly attenuated the K^+ response. Thus, any hypothesis to explain altered neuronal function during hypoxia must include regional differences in cellular calcium regulation and a differential response to calcium antagonists.

665.3  ISCHEMIA-INDUCED REGIONAL ALTERATIONS IN PKC ACTIVITY ARE SENSITIVE TO MODERATE CHANGES IN INTRAISCHEMIC BRAIN TEMPERATURE. R. Bustos, M.L.T. Gobius, E. Martinez, L. Valdez, and M.D. Ginsberg. CVD Research Center, Univ. of Miami, Sch. of Med., Miami, FL 33101.

Extracellular glutamate release and histopathological outcome following ischemia are sensitive to intracranial brain temperature. Since PKC is implicated in neurotransmitter release and glutamate receptor mediated events, the relationship between intracranial brain temperature and PKC activity was evaluated. Twenty min of 2-vessel occlusion plus hypotension was induced in rats whose intraischemic brain temperature was maintained at 37°C, 35°C, or 30°C. Using a PKC enzyme kit, cytosol and membrane PKC activity were determined in hippocampus, striatum, cortical, and thalamic homogenates at the end of ischemia and at 15min, 30min, 1 hr and 24hr of recirculation. A reduction in cytosol and membrane PKC activity (43-50% and 45-49%, respectively; p < 0.05) was observed in the hippocampus, striatum and cortex of normothermic rats as early as 30min of recirculation. PKC activity remained diminished at 24hr in the hippocampus and striatum but normalized in the cortex. No changes were documented in the thalamus. Ischemia-induced reductions in PKC activity were abolished in hypothermic animals. Conversely, postischemic PKC activity was significantly lower in the hippocampus and cortex of hyperthermic animals compared to normothermic (p < 0.01). Results indicate the ischemia-induced changes in PKC activity is a temperature sensitive process, suggesting that it may be involved in the effects of temperature on ischemic outcome.

665.5  EXPRESSION OF PROTEIN KINASE C ISOZYMES AND THE EFFECT OF HYPOXIA ON PKC IN RAT GLIAL CELLS. K. Kumar*, B. Kim, H. Rupp, & B. V. Madhukar. Departments of Pathology, and Pharmacology and Human Development, Michigan State University, East Lansing, MI 48824.

Hypoxia and ischemia produce a number of intracellular changes conducing to the activation of Protein Kinase C (PKC) that has a number of isoforms. To study the presence of various isoforms of PKC in glial cells, the effect of hypoxia on PKC, immunofluorescence (IF) studies were performed on glial cell cultures of the rat cortex. The antibodies tested were against α, β, γ, and ε isoforms, and the catalytic domain (cd), of PKC. The degree of IF for each group of cells was compared by using an automated laser cytometric device. The data indicate that rat glial cells express at least three isoforms of PKC, α, β, and α (in descending order of intensity). The IF for PKC - cd was more intense than for any of the isoforms. The glial cells were subjected to hypoxia by exposure to 100% N_2 for varying periods of up to 72 hr. IF studies were performed as above. Even though this was not a quantitative study, a decrease in the PKC - cd IF was demonstrated following 72 h of hypoxia. Decrease in the activity of PKC may lead to altered protein phosphorylation reactions, thereby playing critical roles in neuronal death due to hypoxia.

665.6  DIFFERENCES IN ALTERNATIONS BETWEEN Ca^2+/CALMODULIN-DEPENDENT PROTEIN KINASE AND CALCINEURIN IN THE HIPPOCAMPUS AFTER TRANSIENT FOREBRAIN ISCHEMIA. M. Morioka*, K. Fukunaga, S. Nagahiro, Y. Ushio and E. Miyamoto. Dept. of Neurosurgery and Pharmacology, Kumamoto University School of Medicine, Kumamoto 860, Japan.

After transient ischemia of the brain, the increase in the intracellular Ca^2+ level is observed, which may trigger the intracellular process of neuronal death. We have investigated the regional and temporal alterations of Ca^2+/calmodulin-dependent protein kinase II (CaM kinase II) and calcineurin (CaN) (Ca2+/CaM-dependent protein phosphatase) after transient forebrain ischemia. The immunocytochemical and enzymatic activity of CaM kinase II decreased in all the regions of the hippocampus at early stage (6-12 hrs) after ischemia, but gradually recovered during the course of the time except for the CA1 region. Furthermore, an increase in Ca2+/CaM-dependent activity was detected at 3 days after ischemia in all regions tested, suggesting that the concentration of intracellular Ca^2+ increases. In contrast to CaM kinase II, immunohistochemistry and regional immunoblot analyses revealed that CaN was more depressed in the CA1 region of CA1 neurons. These results suggest differences between CaM kinase II and CaN in regard to regional and temporal loss after ischemia and the occurrence of the injury, CaM/Ca^2+/CaM-dependent protein phosphatase-dephosphorylation.
REGIONAL DEPRESSION IN CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE II (CaM-KII) IN FOCAL ISCHEMIA. S.K. Hanson, J.C. Grotta, M.N. Waxham, R. Earls, R. Strong, N. Dafoys-. Dept. of Neurology and Neurobiology and Anatomy. Univ. of Texas Medical School, Houston, TX 77030

Change in CaM-KII activity was evaluated in a rat model of focal ischemia. Activity in homogenates prepared from cortex measured by phosphorylation of synthetic peptide substrate was decreased by 76% (p<0.0001) in histologically defined infarct border zone (n=12) when compared with similar cortical regions in sham operated controls (n=4). Decreases in histology-defined infarct borderzone (defined at one hour of ischemia) were less dramatic (34%, p=0.0007). These changes were immediate so that depression in activity after 45 min was measurable to that seen after one hour of ischemia with no reperfusion. Reperfusion is associated with incomplete return of activity at 24 hours even in regions with no apparent histologic damage. CaM-KII downregulation is extremely sensitive to focal ischemic insult and changes in activity are proportional to the amount of histologic damage.


High cytosolic calcium levels are thought to lead to neuronal damage after anoxia. We found that Ca-45 uptake during anoxia was far less than the uptake measured during depolarization. We therefore examined whether anoxia could inhibit depolarization induced Ca uptake.

Hippocampal slices were subjected to either anoxia (ischemia, depolarization, (12.5 µM veratridine) or a combination of the two. Ca-45 uptake was measured in the CA 1 region.

Calcium uptake increased from 9.9 ± 1.2 to 10.7 ± 2.2 nM/mg dry wt. (P < .001) during 15 min of anoxia. Veratridine for 10 min caused a 3 fold increase. Ca-uptake to 30.2 ± 1.7 nM was started 5 min before the onset of veratridine depolarization and continued throughout the depolarization (total anoxia 15 min, veratridine = 10 min) then there was only a 2 fold increase in calcium uptake to 18.4 nM/mg.

Thus anoxia reduced the depolarization induced calcium uptake indicating that there might be a mechanism by which anoxia can inhibit the voltage sensitive calcium channel.

BRAIN FUNCTIONAL ABNORMALITIES IN CHRONIC ESSENTIAL HYPERTENSION. MJ Mennis, JA Galeno, B Howitz. Murphy DGM, St Rapport. MB Schapira, Lab. Neurosci., Nail Inst on Aging, NIH, Bethesda MD.

To determine if chronic essential hypertension is associated with abnormal brain function, we performed a cross-sectional analysis on the results from 17 hypertensive men (mean ages 68±10), and 25 age- and sex-matched normotensive control subjects, who were studied with high-resolution positron emission tomography (PET) using 18-fluoro-deoxyglucose when off medication for at least 2 weeks. Hypertensives had been medically-treated for at least 10 yrs, and had no apparent end-organ involvement. All subjects were free from other medical, and cognitive disorders. A correlational analysis was performed to count the number of significantly different correlation pairs (sdc) between the two groups in the vascular areas of the brain. Correlations were performed from each vascular area of interest to all brain areas. There were significantly more sdc's with a smaller hypertensive value in the carotid distribution compared to the basilar, and within the carotid area, in the middle-anterior watershed area compared to the middle and anterior cerebral artery areas. These results suggest that well-controlled hypertensives without clinical cognitive or other deficits, have abnormal brain correlations in the carotid artery area, and within this territory mostly in the middle-anterior artery watershed area. This latter pattern of functional abnormality - mainly in the watershed area - suggests a low flow state (ies episodes of hypotension) rather than high pressure as the immediate cause.

REGIONAL DEPRESSION IN CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE II (CaM-KII) IN FOCAL ISCHEMIA. S.K. Hanson, J.C. Grotta, M.N. Waxham, R. Earls, R. Strong, N. Dafoys-. Dept. of Neurology and Neurobiology and Anatomy. Univ. of Texas Medical School, Houston, TX 77030

CHANGE IN CALCIUM UPTAKE INDUCED BY DEPOLARIZATION IN THE HIPPOCAMPAL SLICE IS EXTREMELY SENSITIVE TO ANOXIA. S.K. Hanson, J.C. Grotta, M.N. Waxham, R. Earls, R. Strong, N. Dafoys-. Dept. of Neurology and Neurobiology and Anatomy. Univ. of Texas Medical School, Houston, TX 77030

Here we describe effects of Dextrorphan (DEX), the well defined noncompetitive NMDA channel antagonist which also express VDCC antagonistic properties, on changes in activity of both CaM-KII and PKC induced by reversible global ischemia in rats. 15 mg/kg of intravenous DEX, 5 min before induction of 20 min of ischemia, significantly down-regulate of both kinases. The observed protection was detected immediately after ischemia as well as after 24 h of reperfusion. There was no effect of DEX on kinase activity observed after 2 h of reperfusion. Pretreatment, but not posttreatment of animals with DEX subjected to 20 min of ischemia also resulted in histological protection of hippocampal and cortical neuronal tissue.

Electrodes Based on Eth-129 ShowAstrocytic Ca2+ Rises During Spreading Depression. R.P. Kraig* & C.D. Lascola. Dept. ofNeurology, Univ. of Chicago, Chicago, IL 60637.

Spreading depression (SD), a benign yet massive perturbation of brain, transiently converts neurons to reactive species (1). A rise in pH, associated with SD (2), may allow this glial metamorphosis to occur but what might trigger it is unknown. A rise in [Ca2+]i stimulates enhanced anabolism, proliferation, in other cells and thus, might trigger reactive astrocytosis (RA).

Ca2+-ISMs based on ETH-129 were used to determine astrocytic [Ca2+]i changes during SD in anesthetized rats. SD was induced by electrical stimulation and astrocytes were identified by electrophysiologic criteria (2). Ca2+-ISMs had a linear response and sensitivity for changes in pCa2 + to almost 8 and at least 10, respectively, in 100 mM K". Membrane potentials fell from 80-110 mV while [Ca2+]i rose from the 100 nM range by orders of magnitude during SD. Both parameters returned to baseline after SD.

Resting astrocytic [Ca2+]i in the nM range and rise with SD is consistent with that seen in vitro (3) and suggests the validity of this data comparing to previous work (4) (based on ETHE-1001) which may have been erroneous due an excessive Ca2+-leak into cells. Perhaps, a coupled rise in pH, and [Ca2+]i, synergistically increase astrocytic anabolic activity in RA.

ANXIETY DISORDERS FOLLOWING STROKE. CS Castillo, SE Starkstein, JP Fodoroff, TR Price, RG Robinson. Univ. of Iowa Coll. Med., Iowa City, IA 52242

A series of 309 admissions to a stroke unit were examined for anxiety symptoms. Patients were diagnosed with DSM-III-R generalized anxiety disorder (GAD) (symptom criteria) (1). They were divided into groups of no anxiety (39.7%), worried but not fulfilling GAD criteria (33.9%), and GAD (26.9%). Patients were then divided into depressed and non-depressed groups based on the existence of DSM-III major or minor (dysthymic) depression. These groups were not significantly different in their background characteristics or the severity of physical impairment. Anxiety plus depression (74%) of all GAD patients and 19% of all patients) was associated with left cortical lesions while anxiety alone was associated with right hemisphere lesions (X2=4.9, df=1, p=.043). Patients with GAD had a greater frequency of posterior right hemisphere lesions than patients with right hemisphere lesions without GAD (F=3.5, df=2,46, p=.038). Two year follow-up at 3,6,12 and 24 months revealed a stable prevalence of GAD at each of follow-up intervals of approximately 80%. Patients who were initially not worried had a mean prevalence of 18% during the 2-year follow-up. The initially GAD (IGAD) patients had a mean follow-up prevalence of 44% while those that were initially worried had a mean prevalence of 30%. Presence in the follow-up depressed patients was 36.5% while in the non-depressed it was 18%. Initially not depressed IGAD (11% prevalence) patients had no cases on follow-up. Initially depressed IGAD maintained the highest follow-through rates (59, 17,43% for 6, 12, 24 months respectively). New onset GAD at follow-up averaged 58% of all GAD cases seen over 2 years and 80% also had depression. Findings suggest that anxiety disorder (independent of depression) is not related to background of ischemic stroke but is influenced by brain structures injured. Post-stroke GAD has a high incidence.
666.3

Motor Control of the Hand in Humans with Upper Motor Neuron Dysfunction Due to Stroke

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Upper limb voluntary motor control impairment, in particular, the loss of fine
precise movements of the hand and fingers is common following stroke. The
evolution of recovery from stroke has a well described pattern with the majority of
functionalmovement occurring in the first 3 months after onset. Func-
tional outcome to Theiler's murine encephalomyelitis virus (TMEV)-
duced demyelinating disease (TMEV-IDD), a model for human multiple
sclerosis. TMEV-IDD is believed to result from bystander damage to myelin infected cells as part of a delayed type hypersensitivity
response directed against chronic, low level infection of the CNS by
TMEV. We have found BALB/cByJ and BALB/cAnNcr to be
resistant to the development of TMEV-IDD, while BALB/cByJ and
BALB/c are intermediate susceptible. We are attempting to
define the genetic and immunological mechanisms involved in this
differentiation in susceptibility and to determine whether some
resistant strains can be converted to a susceptible phenotype by
exposure to infection with TMEV, of immunopotentiating regimens such as low dose cyclophosphamide or
eradiation or administration of anti-I-J antibodies. Low dose
treatment efficiently converted resistant BALB/cByJ mice to a
susceptible phenotype, indicating that the resistance of BALB/cByJ to
development of the immune response associated with development of
TMEV-IDD is due to an active regulatory mechanism rather than an
intrinsic inability to develop the destructive responses. Low dose
cyclophosphamide was far less effective in producing the susceptibility in BALB/cByJ. Experiments have been initiated to
identify the cellular mechanisms involved in the protective regulation.

666.4


EEG was recorded from 16 normal subjects and serially from 16 patients with
cerebral aneurysms (14 had subarachnoid hemorrhage; 15/16 aneurysms
were surgically repaired). The EEG was quantified (Cadwell Spectrum 32) and
analyzed with regard to absolute and relative power, symmetry, and coherence
over 4 frequency bands and 21 sites. In general, patients showed increased
slowly, frontal power asymmetry, and increased coherence over temporal sites.
Focal abnormalities in data power and coherence were characteristic of an
aneurysmal source in 12 preoperative studies. Anterior communicating artery
aneurysms were linked with tempo-temporal absolute power, while coherence
abnormalities in anterior, central and posterior sites were seen with internal
carotid, middle cerebral and posterior communicating artery aneurysms,
respectively. In 3 patients, QEEG suggested aneurysmal sources at a time
when angiographic data were either negative or inconclusive. Abnormalities were also
seen in patients with incidental aneurysm findings, suggesting that the QEEG
does not simply reflect the presence of SAH. Postoperatively, 9 of 15 patients
developed vasospasm by clinical or radiographic criteria. In 3 of 5 patients who received pre- and post-spasm QEEG studies, novel focal abnormalities in QEEG
could be seen prior to the onset of vasospasm, and the brain regions involved
were appropriate to the type of functional deficit that eventually emerged.
Although preliminary, these results suggest that quantifiable changes in EEG
distribution over the scalp may be useful both in diagnosis of aneurysms in patients with SAH, and in the detection or prediction of impending vasospasm.
Further studies are aimed at determining the reliability and validity of this
technique to detect subclinical cerebral vasospasm and allow early intervention.
This work was supported in part by the Departments of Neurosurgery and
Clinical Neurophysiology at Sentara Norfolk General Hospital.

INFECTIOUS DISEASES

667.1

BALB/c SUBSTRAIN DIFFERENCES IN SUSCEPTIBILITY TO TMEV-INDUCED DEMYELINATING DISEASE AND POSSIBLE MECHANISMS. S.M. Nicholson, T.L. Holler, L.D. Dech, C.Waltenbaugh, and R.W. McEwoll. Dept. of Microbiology and Immunology and the Institute for Neuroscience, Northwestern University, Evanston, IL, 60201.

We report differences among four BALB/c substrains in susceptibility to
TMEV-induced demyelinating disease (TMEV-IDD), a model for human multiple
sclerosis. TMEV-IDD is believed to result from bystander damage to myelin infected cells as part of a delayed type hypersensitivity
response directed against chronic, low level infection of the CNS by
TMEV. We have found BALB/cByJ and BALB/cAnNcr to be
resistant to the development of TMEV-IDD, while BALB/cByJ and
BALB/c are intermediate susceptible. We are attempting to
define the genetic and immunological mechanisms involved in this
differentiation in susceptibility and to determine whether some
resistant strains can be converted to a susceptible phenotype by
exposure to infection with TMEV, of immunopotentiating regimens such as low dose cyclophosphamide or
erythromycin or administration of anti-I-J antibodies. Low dose
treatment efficiently converted resistant BALB/cByJ mice to a
susceptible phenotype, indicating that the resistance of BALB/cByJ to
development of the immune response associated with development of
TMEV-IDD is due to an active regulatory mechanism rather than an
intrinsic inability to develop the destructive responses. Low dose
cyclophosphamide was far less effective in producing the susceptibility in BALB/cByJ. Experiments have been initiated to
identify the cellular mechanisms involved in the protective regulation.

667.2


Supported by USPHS grant NS 16102.

There is mounting evidence that inflammation plays a role in the pathogenesis of multiple sclerosis. TMEV-induced demyelinating disease (TMEV-IDD) in the nigrostriatal system is believed to be associated with a transient inflammatory response. In this study, we examined the role of the cytokines IL-1 and TGF-B1 in the pathogenesis of TMEV-IDD in the rat striatum.

IL-1 and TGF-B1 are produced by microglia and astrocytes in response to injury and infection. In the present study, we have demonstrated that injection of IL-1 and TGF-B1 into the striatum of rats results in an increase in the number of astrocytes, as measured by immunohistochemistry. This increase in astrocyte number is greatest in the striatum, but is also seen in the substantia nigra and the entorhinal cortex.

Furthermore, we have found that injection of IL-1 and TGF-B1 into the striatum of rats results in an increase in the number of astrocytes, as measured by immunohistochemistry. This increase in astrocyte number is greatest in the striatum, but is also seen in the substantia nigra and the entorhinal cortex.

In conclusion, our results suggest that IL-1 and TGF-B1 play a role in the pathogenesis of TMEV-IDD in the rat striatum.
GENETICALLY DETERMINED HERPES SIMPLEX VIRUS 1 (HSV 1) RESISTANCE IN OLIGODENDROCYTES (OL) MAY PLAY A ROLE IN LIMITING SPREAD OF HSV 1 IN THE CENTRAL NERVOUS SYSTEM (CNS): DELAY OF IMMEDIATE EARLY (ICP4) AND EARLY (ICP8) ANTIGEN SYNTHESIS IN HSV 1 RESISTANT OLs FROM C57BL/6J AND BALB/C BY MICE. E.E. Thomas*, A. Labin, I.F. Estes,§ Division of Microbiology, Department of Pathology, British Columbia Children's Hospital and 2 Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, Canada.

Primary murine OL cultures from 3 inbred strains show HSV 1 replication differences, which correlate with in vivo infection (AD susceptible; BALB/cJ, moderately resistant; C57BL/6J, resistant). The nature of the in vivo resistance at the cellular level has yet to be determined. Immunofluorescence assay (IFA) with monoclonal antibodies to ICP4, ICP8 and gC HSV antigens. OLs from HSV C57BL/6J mice showed restricted expression of all antigens: only 1% ICP4 (10% of cells) and no gC was detected until 72 hrs postinfection (p.i.) in contrast to OLs from AD mice in which 80-100 % of cells expressed all three proteins. 72 hrs p.i. Balb/c showed an intermediate protein expression pattern: 50% of cells were ICP4 pos., 30-50% ICP8 pos. and 20% gC pos. 72 hrs p.i. HSV adsorption (using [3H] labelled HSV) to the 3 OL strains was also studied, and no difference in adsorption pattern was noted. These results suggest the existence of an early, postranslation HSV replicative block in C57BL/6J OLs and a less restricted early replication block in Balb/cJ OLs.

Electrophoresis studies show no detectable differences in HSV resistance mediated by OLs, reflect differences in virus host cell interactions, and likely contribute to differences in mortality, viral spread, and pathologic differences in the CNS of the different mouse strains.

Societies for Neuroscience Abstracts, Volume 18, 1992

667.5

Lentiviruses including VISNA in sheep and HIV in humans cause neuronal damage in spite of their not having been seen to replicate in neurons. We have studied the time course and dose dependence of neurotoxicity following direct injection of amino-acid sequences from proteins derived from both viruses into the striatum of rats.

We estimated the neurotoxic effect by measuring the volume of reactive microglial immunostaining in the striatum one week after the toxin injections. The minimal dose required to elicit a measurable lesion was between 1.5 5 μg (0.8 - 4nM) of the peptide from VISNA.

Prominent results suggest that while the cystine residues can modify the change in toxicity, the arginine doublet in the centre region is necessary. Since these peptides derive from the toxic sequences in the viral peptides and are thus expected to be passed on to neighbouring cells, secretion of them by infected glia could both damage nearby neurons and recruit more microglia thus explaining the neuropathological pattern seen in infected animals.

667.6
NEUROTROPHIC FACTORS MEDIATE NEURONAL DEATH IN MOUSE SPINAL GANGLIA FOLLOWING HERPES SIMPLEX VIRUS TYPE 2 (HSV-2) INFECTION. R. H renken1, M.E. Goldstein2, A.R. Martin3, NIH, NINDS, LEAD, NICHD, Bethesda, MD, USA, 20892.

In this study, we examine whether HSV-2 infection causes neuronal death in mouse dorsal root ganglia (DRG). The right hind footpads of anaesthetized female BALB/c mice were inoculated with 10 μl of strain HSV-2 (9.3x10^6 pfu/ml) or medium. One month later, infected (+) and sham-inoculated (+) mice were perfused and examined. Spinal columns, including paired spinal ganglia, were stained with cresyl violet. Neuronal number, somal areas and ganglion volumes for both the infected and the contralateral uninjected lumbar 4 and 5 DRG were determined for each mouse following HSV-2 infection. Between 50 and 70% of neurons disappeared 1 month following inoculation. Neuron numbers in sham-inoculated and contralateral control ganglia were equivalent. Neuronal death did not target a specific subpopulation of neurons, based on somal size. Ganglionic shrinkage did not occur as a result of HSV-2 infection; neurons were replaced by large numbers of inflammatory cells. These results show that, in addition to other previously described host alterations following viral infection, HSV-2 infection results in neuronal death in the affected ganglia. This is the first in vivo quantitative documentation of HSV-2 induced neuronal death

667.7
VIRUS DNA SEQUENCES AND CNS DISEASE IN NEONATAL HERPES SIMPLEX INFECTION. P. Greer1, C. Langston*, W. J. Mitchell' and J. R. Martin2* NIH, Bethesda, MD; 2Texas Children's Hospital, Houston, TX

This study used direct cytoplasmic detection (PCR), a test to detect herpes simplex virus (HSV) genomic sequences in formalin fixed paraffin sections from perinatal HSV-1 and -2 encephalitis autopsy tissues and correlates these results with immunohistochemistry (IHC) and histological lesions. Brain sections from 10 mice with acute HSV-2 infection and 20 uninfected controls were used to establish PCR conditions of specific HSV DNA amplification and detection. Primers which bracketed a 110 BP fragment of the HSV-2 thymidine kinase gene, 30 cycles of amplification and Southern blot hybridization with an appropriate oligonucleotide probe were selected as they gave 100% sensitivity and specificity in repeated tests with control mouse tissues. 56 neuronal and non-neuronal tissue (N-NT) blocks from 10 neonates were tested; previous IHC studies showed that in N-NT of 6 neonates antigen contained HSV DNA sequences, confirming the sensitivity of this PCR protocol. In NT, 22 blocks displayed histological lesions of possible viral origin but the IHC detected HSV antigen in only 9 blocks; in contrast, we obtained a specific band of amplified HSV DNA in all these blocks, including 1 neonate dying 7 weeks after birth. 15 NT and N-NT blocks were histologically normal and HSV antigen-negative; all except 2 blocks from the 7 week old neonate, contained HSV DNA sequences. All PCR results were reproducible. PCR detects HSV DNA in CNS areas that contain no detectable HSV antigen but are histopathologically abnormal; this suggests that these histological lesions are virus-induced.

667.8
ANALYSIS OF MYELIN PROTEINS IN POST MORTEM AIDS BRAIN. J.R. Möller, P.G. Durr, R.H. Quarles, B.D. Trapp, C. Power and J.T. Kastrukoff2* 1 Division of Microbiology, Department of Pathology, British Columbia Children's Hospital, Vancouver, Canada; 2Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, Canada.

Subcortical white matter samples from five AIDS patients and four age matched controls were histochromically and biochemically characterized for myelin proteins. The pathology observed histologically in the AIDS patients included diffuse subcortical white-matter pallor, as visualized by Luxol Fast Blue staining. Subcortical white matter samples from five AIDS patients and five controls. Samples for biochemical analysis were adjacent to areas of HSV antigen but are histologically abnormal; this suggests that these histological lesions are virus-induced.

667.9
HYPERGLIAL REACTIONS IN HIV-1 INFECTION. P. Gao, L. Yang, L. Zhang, Z. Zhou, D. Henken*, M.E. Goldstein, and J.R. Martin, NIH, NINDS, LEAD, NICHD, Bethesda, MD, USA, 20892.

Hypervascular brain tissue was studied in HIV-1 infected individuals as compared to controls. Electron microscopy of subcortical white matter from HIV-1 infected individuals showed hyperplasia with a focus of demyelination.

Samples for biochemical analysis were adjacent to areas examined histologically and were homogenized in 1% SDS and boiled for 10 minutes. Controls were blue stained gels and Western blots were utilized for quantification of MBP, PLP, MAG, 2',3' cyclic nucleotide 3-phosphodiesterase (CNP) and GPAP. No major quantitative differences were found in the samples from AIDS patients were found. These results do not provide morphological or biochemical evidence for extensive myelin loss in subcortical white-matter of AIDS patients.

We describe the occurrence of histologic lesions within the central nervous system of monkeys following infection with HIV-1 virus, the etiologic agent of human AIDS. Eighteen adult pigtailed macaques (Macaca nemestrina) were inoculated intravenously with a mixture of infected autologous peripheral blood mononuclear cells (PBMC) and free virus of three HIV-1 strains: LAI, JR-CSF, and JR-FL. A comparison group was infected with SIV. At 2, 5, 14, and 24 weeks following infection, animals were killed and examined histologically for lesions in the central nervous system.

Initial examination of the brains of two animals sacrificed 5 weeks after infection with HIV-1 showed choroiditis in both animals and extensive perivascular infiltrates in the occipital cortex of one. Choroidal infiltrates were composed predominately of lymphocytes; perivascular infiltrates were composed primarily of cells of macrophage origin with occasional astrocytes present. HIV-1 was isolated from PBMC's taken at the necropsy of each animal.

Tests for virus in the tissues are being conducted by immunocytochemistry of the macrophage species following infection with HIV-1.

(Supported by USPHS grant RR00166 and Comparative Medicine Training Grant RR07019.)


Asymptomatic HIV seropositive subjects often exhibit evidence of CNS infection with HIV. Progression of the infection with HIV in the brain is suggested by the clinical manifestations of neurological disorders of AIDS dementia complex. The factors that could modulate HIV replication in the brain have not been investigated. We investigated the effects of cytokines on HIV transcription in human glialoma cells (1321N1) and primary rat astrocyte cultures, as well as the effect of substance P (SP) on HIV transcription and IL-1 production in human macrophage cells. HIV-LTR was linked to the cDNA of chloramphenical acetyl transferase enzyme (CAT) and increased synthesis of CAT enzyme transcription was considered indicative of HIV transcription. The results indicate that: 1) SP dose-dependently enhanced HIV transcription in the macrophage, with the minimal effective dose at $10^{-10}$ M and SP also induced the synthesis of IL-1; 2) IL-1 (10-100 pg/ml) and TNFa (100-1000 pg/ml) significantly enhanced HIV transcription; and 3) IL-1 and TNFa in concert with each other synergistically enhanced HIV transcription. These results suggest that neural and immune mediators may promote the progression of HIV infection in the brain and contribute to neuropathology of AIDS.


Lab. of Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20892; Lab. of Neurochem. NINDS; NIH; Dept. of Biochem., George Washington Univ. School of Med., Washington D.C. 20037.

Previous studies have shown that the purified envelope protein gp120 from the human immunodeficiency virus produces neuronal cell death in hippocampal cultures derived from fetal mice (Brenneman et al., Nature 289: 345, 1988). Subsequent studies have indicated that cortical neurodegeneration and delays in developmental milestones occur in developing rats administered gp120 (Hill et al., Soc. Neurosci. Abstrs. 16: 615, 1990). Purified gp120 was radiolabeled and injected subcutaneously into one-day-old rats. The pups were either freeze and sectioned for in vivo autoradiography, or the brains were homogenized and assayed by FPLC for the presence and distribution of labeled gp120 and its proteolytic fragments. In vivo autoradiography revealed that radioactive material reached the brain and diffused into adjacent brain tissue. FPLC analysis revealed four major peaks of radioactivity. The chromatographic fractions corresponding to these peaks were assayed for neurotoxicity on dissociated cortical cultures. All four peaks exhibited neurotoxic activity that could be prevented by co-treatment of the test cultures with 1 mM peptide T. These data suggest that low molecular weight (<800 Dalton), neurotoxic gp120 fragments may play a role in the etiology of "Neuroides".


AIDS often is accompanied by progressive encephalopathy resulting in 'subcortical' dementia, but it is uncertain how early in HIV infection the brain involvement may begin. This study recorded EEG and ERP (auditory oddball) data from healthy controls and from patients at worsening stages of HIV infection. Neither digital frequency analysis nor nonlinear dynamical (chaos) analysis of the EEG showed differences between healthy controls and any patient group. ERP sensory components also did not differ between groups, but cognitive components showed progressive delays and amplitude reductions corresponding to increasingly severe clinical stages of HIV infection. The earliest changes were among asymptomatic HIV+ patients, suggesting that this test is a sensitive indicator of early subclinical CNS damage and may be of value in decisions regarding early aggressive antiviral therapy and in monitoring its effectiveness.

667.15 ENHANCED CELL MEMBRANE TRANSPORT AND ANTI-HIV-1 ACTIVITY OF ANTI-REV ANTIBODIES FOLLOWING CATIONIZATION. W.M. Parse, D. T. Jackson, U. Bickel, L. Bucik, L. Yang, and C. Marcham*, Departments of Medicine and Neurology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The replication of the human immunodeficiency virus (HIV) is acquired immune deficiency syndrome (AIDS) is dependent on the function of the rev protein. In order to develop anti-rev antibodies capable of transport against a 16-amino acid synthetic peptide encompassing the active site of the rev protein, we developed a new method to cationize antibodies that is rapid, simple and sensitive. Using synthetic peptide, and radioimmunoassay experiments showed that the affinity of the anti-rev antibody for the synthetic peptide was virtually unchanged following site protected cationization. The cationized native anti-rev antibodies were radiolabeled and the cationized antibodies were found to undergo enhanced uptake into either human lymphocytes or bovine brain capillaries as compared to the native anti-rev antibodies which showed minimal cellular uptake. Cationized anti-rev antibodies, but not native anti-rev antibodies or cationized nonspecific antibodies, resulted in a 36% decrease in "H-thymidine incorporation in the human lymphocytes, but a 90% decrease in HIV-1 replication in human peripheral blood lymphocytes grown in primary tissue culture.


In an attempt to find compounds capable of inhibiting the binding and infection by HIV in neural cells, we studied the effect of benzopurpurin and related compounds on the binding of gp120 to GalCer and sulfatide. By using an HPTLC binding assay, we show that the binding of gp120 to GalCer and sulfatide is inhibited by benzopurpurin and related compounds. These compounds also inhibit the binding and entry of HIV into the neural cell line, SK-8-MC. We also show that this method can be used for screening of potential anti-HIV compounds.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992 FRIDAY AM

Membrane permeability and intracellular Ca accumulation plays a fundamental pathogenetic role in hereditary muscular dystrophy. To study the dystrophic muscle metabolism, glycogen, glucose-6-phosphate (G6P), hexokinase (HK), G6PDH, pyruvate, lactate, and creatine phosphate (CP) were quantitated in ventricular myocardium (VM) and rectus femoris (RF) muscle of CHF-146 normal albino hamsters (NH) and CHF-146 dystrophic hamsters (DH) with hyperangiopathy. We observed a marked decrease in energy states aconitase (AcO), CPK, and aldolase (ALD) consistent with an increase in HK and G6PDH in VM and RF of DH. The stimulation of HK and G6PDH, with reduced glycogen and G6P, highlighted the shift of muscle metabolism in DH towards the pentose shunt pathway (PSP). Unaltered pyruvate and lactate levels, together with lower G6P in dystrophic RF (79%) and VM (70%), strongly suggest that the metabolic path in DH is selectively favored towards PSP for the generation of NADPH, reentering the glycolytic pathway at the site of glyceraldehyde-3-phosphate, and thus conserving one ATP in the process. A 75% decrease of G6P in dystrophic VM, compared to a 22% decrease of ATP, suggests that CrP acts as a buffering system for the maintenance of ATP. These findings concur with our report of decreased cellular energy charge in DH (J. Neurol. Neurosurg. Psychiat. 57:1609, 1991). We conclude that muscle metabolism in DH is directed towards the conservation and generation of ATP through the CrP/Cr system, due to an increased energy demand to pump excessive intracellular Ca2+ from the sarcoplasmic reticulum and sarcoplasmic reticulum Ca2+ ATPase. This may contribute to marked depletion of energy rich biomolecules and progressive muscle wasting in DH. (Supported by NIH Grant R01-AR38540)


Intramyofiber VM, a cytoskeletal intermediate filament protein, is expressed in fetal myotubes during myogenesis, presumably important in their early development. As maturation proceeds, desmin replaces VM in the myofibers. In normal mature muscle, VM reappears in myofibers regenerating following injury or disease. We studied VM mRNA expression in biopsied muscle samples of normal(1), Duchenne muscular dystrophy(DM,1), spinal muscular atrophy(SMA,1), sporadic adult-onset myotubular myopathy(SAMMM,1) and polymyositis(PM,2). 10u cryosections were hybridized in situ with a 35-S labeled cRNA probe and analysed by emulsion autoradiography. Serial sections were stained with the modified trichrome. All samples showed VM mRNA in Schwann cells, fibroblasts and blood vessels. In normal animal, no intramyofiber VM mRNA was seen. In contrast, intramyofiber VM mRNA was seen in i) regenerating-degenerating myofibers in DM and SMA, ii) a few myofibers with central nuclei in SMA, VM expression in diseased myofibers suggests a VM-associated regenerative phenomenon, which may be amenable to therapeutic manipulation. (Supported by the Dept. of Veterans Affairs & partly by NIH GM01337 to SSS)

668.5 THE PROGRESSION OF MOVEMENT DISORDERS IN PATIENTS WITH RSD. C.J. Hunkele* and M. Backonja. Dept. of Neurology, U. of Wisconsin, Madison, WI 53792-3493.

Neuropathic pain syndromes with sympathetic involvement traditionally known as reflex sympathetic dystrophy (RSD) often present associated muscle weakness, tenderness, dyskinesia, dystonia, and synkinesis as primary movement disorders in affected limbs (Schwartzman & Kerrigan, 1990; Hunkele and Backonja, 1991). These data supported a CNS pathogenesis; a formally contested issue. The progression of pain features to 'non-affected' body parts has been anecdotally reported in humans and demonstrated empirically in animal models. A similar course for movement control aberrations has not been investigated. Maximal and controlled voluntary contractions were examined in 25 RSD patients via isometric wrist and thumb/index finger force control. Movement control abnormalities in the form of tenderness, dyskinesia, and dystonia were also recorded from contralateral limbs in 80% of the patients. The progressive involvement of contralateral limbs with the RSD-associated movement disorders further support the putative CNS pathophysiological substrate.


Effects of intrathecal baclofen were studied in six patients with spasticity due to spinal cord injury and two subjects with hemiparesis and hemichorea due to stroke. Intrathecal baclofen was ineffective in another brain trauma subject. It did not induce any negative changes in the voluntary motor performance in both "good" and "bad" sides of this subject. The subject displayed similar peak speeds, ranges, and EMG patterns before and after the drug. We hypothesize that adaptive changes in the spinal cord to a traumatic lesion include an increase in the number and/or affinity of the GABA-ergic receptors leading to the selective action of intrathecal baclofen.


Generation of energy rich biomolecules such as ATP and creatine phosphate (CrP) is crucial for physiologic functioning of heart and skeletal muscle. Because of our recent findings of diminution of ADP phosphorylation and glycogenolysis, we investigated some of the key enzymes of ADP phosphorylation, glycogenolysis, pathway, Krebs cycle, and mitochondrial respiratory chain. Biochemical assays were performed in post 10,000 g supernatants of ventricular myocardium (VM) and rectus femoris (RF) muscle of CHF-146 normal albino hamsters (NH) and CHF-146 dystrophic VM, a significant decrease in the activity of glycogenic enzymes like pyruvate kinase (PK), aldolase (ALD) and lactate dehydrogenase (LDH) was observed by 29%, 39% and 37%, respectively. Activity of creatine phosphokinase (CPK) was reduced by 37% in dystrophic VM. Several mitochondrial key enzymes such as succinate dehydrogenase (SDH), citrate synthetase (CS), NADH-cytochrome-c-reductase (NCCR), succinate-cytochrome-c-reductase (SCCR) and NADH-ferri cyanide reductase (NFR) were also reduced in dystrophic VM by 34%, 31%, 27%, 52% and 19%, respectively. Similarly, dystrophic RF revealed a significant lower PK (34%), LDH (29%), ALD (47%), CPK (33%), aldolase kinase (33%), SDH (37%), CS (29%), NCCR (48%), SCCR (21%) and NFR (23%), compared to NH. These data strongly suggest a severe impairment of glycolytic pathway, as well as mitochondrial energy metabolisms at the level of the Krebs cycle and electron transport chain system in dystrophic VM and RF, contributing to marked depletion of energy rich biomolecules and progressive muscle wasting in DH. (Supported by NIH Grant R01-AR38540)


Effects of intrathecal baclofen were studied in six patients with spasticity due to spinal cord injury and two subjects with hemiparesis and hemichorea due to stroke. Intrathecal injection of baclofen effectively suppressed spastic signs in all the spinal cord injury patients. Spasticity decreased rapidly and was associated with a significant decrease in voluntary movement and walking distance. These effects were accompanied by a general improvement of the patterns of muscle activation. The inhibition of the co-contraction and decrease in the antagonist and distant muscle groups. The decrease in spasticity was accompanied by a general improvement of the patterns of muscle activation. The inhibition of the co-contraction and decrease in antagonist and distant muscle groups. The decrease in spasticity was significantly lower PK (34%), LDH (29%), ALD (47%), CPK (33%), aldolase kinase (33%), SDH (37%), CS (29%), NCCR (48%), SCCR (21%) and NFR (23%), compared to NH. These data strongly suggest a severe impairment of glycolytic pathway, as well as mitochondrial energy metabolisms at the level of the Krebs cycle and electron transport chain system in dystrophic VM and RF, contributing to marked depletion of energy rich biomolecules and progressive muscle wasting in DH. (Supported by NIH Grant R01-AR38540)
668.7

POSTURAL STABILITY AND VARIABILITY IN TARDIVE DYSENKINESIA.
Department of Kinesiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

Stereotypic motions are a characteristic feature of
tardive dyskinesia (TD). These motions can arise in a
variety of tasks including bi-pedal posture. This study
examined the structure of the center of pressure profile in 3
groups (rhythmic TD, non-rhythmic TD, normal
control) of trialls. Our findings suggest that: a) there
is structure to the usually constant random pattern of
the "normal" center of pressure; b) that some of the
developmentally disabled tardive dyskinetic subjects
exhibited a cyclical center of pressure pattern that
is consistent with a limit cycle attractor organization; c)
normal center of pressure has a higher dimensionality; and
d) that variability of the center of pressure can only be
interpreted as reflective of stability when the same
attractor dynamics are evident in organizing the posture.
These findings suggest: (1) that center of pressure vari-
ability needs to be interpreted in relation to dimension-
ality of the supporting dynamic; (2) significant limita-
tions to traditional interpretations of the relation
between center of pressure variability and posture stabili-
ty; and (3) new ways to analyze human postural dynamics.

668.8

BROAD A BAND DISEASE: A NEW CONGENITAL MYOPATHY
ASSOCIATED WITH LEBER'S CONGENITAL AMAUROSIS.
R. E. Mkrl1, B. Lange, and M. C. Rodsky.
Departments of Pathology, Pediatrics, Neurology and Ophthalmology; Univ.
Arkansas Medical Sciences and VAMC, Little Rock, AR 72205.

A two-year-old boy with Leber's congenital amaurosis displayed
diffuse hypotonia with delayed motor milestones, depressed deep
tendon reflexes and normal sensation. Histological and
histochemical evaluation of biopsied thigh muscle showed no
abnormality. One-µm plastic sections and electron microscopy
showed numerous foci of broadening or smearing of the A-band
with loss of a distinct I band. Z-lines were normal except for a fine
waviness in these areas. The inter-Z-line sarcotubular had a staining
density intermediate between normal A and I bands, and the thick
filaments in these lesions appeared misaligned. The smaller lesions
involved a single sarcotubule of a single myofibril, while larger
lesions extended laterally across 3-4 myofibrils and longitudinally
along 3-4 sarcomeres. There were 24 such lesions in 10 random
2400 µm² fields, but none in 9 other hypotonic patients with
"unstructured" myopathies. These findings suggest an abnormality
of the M-line or of the structural protein connectin. These findings
differ from those of previously-described congenital myopathies,
and represent the first described morphological abnormality of
muscle in a patient with Leber's congenital amaurosis.

668.9

ULTRASTRUCTURAL PATHOLOGY OF SKELETAL MUSCLE IN PROGRESSIVE
SYSTEMIC SCLEROSIS, A. Márquez, H. Rivera, H.J. Fink1
1, Montes de Oca and B. Molnar. Instituto de Experimental
Medicina, Universidad Central de Venezuela, Apartado 50587,
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Upon careful examination the majority of patients with progressive
systemic sclerosis (PSS) are found to have mucosal proximal weakness and wasting, elevation of
plasma level of muscle enzymes and alterations in EMG. Using
light microscopy two different pathologic entities have
been described, simple myopathy and myositis. Ultrastruc-
tural studies on the muscle lesions have emphasized the
vascular changes. In this work we report the whole spec-
trum of changes observed by electron microscopy in the
study of needle muscle biopsies from five patients with
positive diagnoses of PSS and a muscle compromise.
The changes observed were: a) a varied degree of fiber atrophy,
fiber necrosis, mitochondrial alterations, vacuolization of
sarcotubular system, lysosomal proliferation, presence of
filamentous and concentric laminated bodies, capillary
alterations included hypertrophy of endothelium with lumen
occlusion, autophagic vacuoles and widening and redupli-
cation of basement membrane, Cell infiltration included
lymphocytes, macrophages and mast cells, This work in-
dicates a multifactorial pathogenesis, neural, vascular
and autoimmune for the muscle damage in PSS.

This work was supported by COCH of UCV ( 03-2709/92),
Fundación Polan and The British Council Venezuela.

668.10

ELECTROMYOGRAPHIC CHANGES COMPARING MEDIAL AND
LATERAL SURGICALLY TRANSFERRED RECTUS FEMORIS MUSCLE IN
CHILDREN WITH CEREBRAL PALSY.
Department of Kinesiology, University of Illinois at Urbana-Champaign,
Urbana, IL 61801.

In 3 groups (rhythmic TD, non-rhythmic TD, normal
control) of trials. Our findings suggest that: a) there
is structure to the usually constant random pattern of
the "normal" center of pressure; b) that some of the
developmentally disabled tardive dyskinetic subjects
exhibited a cyclical center of pressure pattern that
is consistent with a limit cycle attractor organization; c)
normal center of pressure has a higher dimensionality; and
d) that variability of the center of pressure can only be
interpreted as reflective of stability when the same
attractor dynamics are evident in organizing the posture.
These findings suggest: (1) that center of pressure vari-
ability needs to be interpreted in relation to dimension-
ality of the supporting dynamic; (2) significant limita-
tions to traditional interpretations of the relation
between center of pressure variability and posture stabili-
ty; and (3) new ways to analyze human postural dynamics.

This was supported by COCH of UCV ( 03-2709/92),
Fundación Polan and The British Council Venezuela.

668.11

SPONTANEOUS RIGIDITY RECORDED IN A MUTANT RAT (TAEIP)
WITH IMMObILITY EPISODES. J. Valencia and J. Acevedo*, Dept.
de Fisiología, Biofísica y Neurociencias, CONEXH-IPN,
México, D.F., and Centro de Invest. en Ciencias Físicas,
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Here we tested whether the immobility episodes of TAEIP
rats were associated with muscular rigidity in both Flexor
and extensor muscles, and whether the rigidity was related to
a decrease in striatal dopamine (DA). To verify this,
the spontaneous electromyographic activity of the Gastro-
emius-Soleus (GS) and Tibialis-Anterior (TA) muscles was
recorded in the mutant rats (n=4), and also, during the
immobility episodes. DA was assayed by HPLC. Both muscles
showed spontaneous tonic activity periods lasting more
than 8 min., in duration. The average firing frequency for
GS was of 21.8±4.5 Hz and for TA, 38.6±5.9 Hz. This tonic
activity was not detected in any animal of the control
group (n=5). During the immobility the firing frequency of
the muscular fibers increased to more than 100 Hz
returning to basal levels afterwards. The high firing fre-
quency was observed only during the immobility episodes.
DA content in the mutant rats (n=6) was 55% higher than
the age-matched controls (n=6). In conclusion, the immo-
Bility episodes were associated with muscular rigidity in
both Flexor and extensor muscles, but the rigidity was
not accompanied by a decrease in striatal DA.

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669.3

Although Beriberi and Wernicke's serve as primordial examples of deficiency disease, the symptoms observed in these conditions manifest themselves only after a severe and protracted vitamin BI depletion. Fatigue is the most common symptom in those cases with mild thiamine deficiency. It is therefore noteworthy that both thiamine deficiency and fatigue are frequently seen in the psychiatric patient population. This study explores for the presence of thiamine deficiency in depression, a disease often characterized by loss of energy and chronic tiredness. Thirty-one patients with major depression (DSM IIIR criteria) and 13 age-matched controls participated in the study. Physical examination and laboratory screening tests were used to identify any concomitant medical conditions and confounding nutritional abnormalities. Erythrocyte homolysates were analyzed spectrophotometrically for the activity of NADH-dependent transketolase both in the presence (TPP) and absence (TK) of thiamine pyrophosphate. Repeated blood laboratory screening tests were used to identify any concomitant medical conditions and confounding nutritional abnormalities. Surface rendering and cross-correlation calculations were performed on a MacIIc with readily available software. The cross-correlation maps were computed by means of a fast Fourier transform (FFT) algorithm. Our results indicate that the cross-correlation technique is reliable and useful for comparing gyral and sulcal patterns between brain regions and across individuals. The cross-correlation results were found to discriminate twin pairs from unrelated individuals more accurately than visual assessment of the same rendered images by 8 raters experienced in MRI analysis. This method may also have broader application in comparing images derived from a variety of modalities, e.g., microscopy, autoradiography, PET, etc.

669.4

Manic states are relatively common among geriatric psychiatric inpatients as well as among younger adult patients. While lithium salts are the first line treatment across the age spectrum, the extent to which optimal treatment conditions change with age has not been adequately investigated. We have therefore begun to study geriatric and younger adult inpatients treated with lithium salts. Twenty-four (n=24) manic patients meeting Feighner criteria for mania were monitored prospectively. They ranged in age from 23 to 89 years and were predominantly female. Manic Rating Scale (MRS) scores declined over 3 weeks of lithium treatment. Greater decreases in MRS were associated with higher plasma lithium levels (p<0.02). Patients with lower Dementia Rating Scale scores had higher MRS scores (p<0.02). These preliminary findings suggest that concentration-effect relationships for lithium exist across the age spectrum. The implications of cognitive dysfunction for management of manic psychopathology require further study. (MBA-2522-01A2)

669.5

The effects of systemic administration ofiriprodole, clinically effective antidepressant which does not inhibit amine uptake or exhibit monamine oxidase inhibitor activity in vitro, on the in vivo extracellular concentrations of NA, DA and 5-HT were examined by brain microdialysis in the medial prefrontal cortex of freely moving rats. Iprindole treatment induced substantial increases in NA and DA outputs in a dose-dependent manner within the range of iprindole tested (25-50 mg/kg). The 5-HT efflux did not change after iprindole administration. The results suggest that iprindole increases availability of synaptic NA and DA in vivo by the different mechanism from the inhibition of amine transport or catabolism. Therefore the results are consistent with the possibility that iprindole enhances the release mechanism of NA and DA, or that there are unknown metabolites of iprindole with amine uptake-blocking potential. Although further study is needed to clarify these possibilities, this finding is consistent with the hypothesis that antidepressants that increase synaptic NA or DA levels cause
AFFECTIVE DISORDERS

FRIDAY AM

1596

Washington Univ. Sch. of Med., St. Louis, MO 63110.

this experimental task reflects the cognitive and emotion-

BLOOD FLOW IN FAMILIAL PURE DEPRESSIVE DISEASE.

3 of 6 normals scanned while thinking sad thoughts were

cases (Wilks' lambda=.42, F=9.5, p<.0001). Of 4 additional

discriminatory capability of PET measurements of regional

thoughts" relative to the "rest" scan, BF increased in the

cortex, amygdala, and related parts of the striatum,
decreased BF in the 1. medial caudate in familial pure

subjects with FPDD (not previously studied) this covar-

al cortex, amygdala, and medial thalamus, and

correctly classified in the FPDD category. In the "sad

incorrectly classified in the FPDD category. In the "sad

subjects with FPDD (not previously studied) this covar-

of FPDD. Discriminant analysis of the covariance of activ-

pallidum, and thalamus is involved in the pathophysiology

directly in various brain regions from subjects with bipolar affective disorder.

guanine nucleotide regulatory (G) protein in bipolar affective disorder which may be

across these cortical regions. The findings of increased cerebral cortical G,

α

significance. A significant correlation (r=0.60) was observed between forskolin-

production was increased to a similar extent but failed to reach statistical

in contrast, no significant differences were found in the other G-protein

subunits measured. Forskolin-stimulated cAMP production was significantly

increased in temporal (p<0.05) elevated in prefrontal (p<0.38), occipital (p<0.80) and temporal

(bottom) cortex but not in hippocampus (p<0.24), thalamus (p>0.23) or cerebellum (p>0.22).

In Western blotting in postmortem brain regions obtained from 10 patients with

Dopaminergic agonists and have examined the behavior and neuroanatomy

schizophrenia(s) are related to errors in fetal development. To test

11-14 of pregnancy with d-araphetamine sulfate (5 mg/kg) or saline.

for NOS using NADPH and nitroblue tetrazolium. The number of NOS-

patterns were examined in a subset of patients. No significant

values peaked 1.81 hr earlier than controls and this difference was

although the unexplained variance in patients was significantly

Although depressed geriatric patients with onset of major de-

comparison with our control, the patterns in all four groups were 

compared with our control, the patterns in all four groups were 

by a sinusoidal function (24 hr fixed period) in both groups 

fit by a sinusoidal function (24 hr fixed period) in both groups 

for Medical Sciences, Little Rock AR 72205.

patterns were examined in a subset of patients. No significant

patients typically have aberrant circadian noradrenergic regulation

rhythms. The effects of chronic desipramine treatment on diurnal

patterns of the noradrenergic metabolite 3-methoxy-

4-hydroxyphenylglycol (MHPG) were studied in the plasma of 43

depressed patients and 12 controls. Daily rhythms were adequately

fit by a sinusoidal function (24 hr fixed period) in both groups although the unexplained variance in patients was significantly 

higher than in controls. Disordered rhythms, while characteristic of the depressed patients, showed no evidence of state dependency. 

Peak MHPG levels, in controls, occurred at 2:54 PM. Patient values peaked 1.8 hr earlier than controls and this difference was 

significant. No group differences were found in either the time independent average levels or sinusoidal amplitudes of the MHPG 

rhythms. The effects of chronic desipramine treatment on diurnal 

patterns were examined in a subset of patients. No significant 

change was found in any variable after treatment regardless of the 

degree of clinical response. The results suggest that depressed 

patients typically have aberrant circadian noradrenergic regulation and 

to a lesser extent either an increased zeitgeber sensitivity or decreased 

circadian period. None of these abnormalities, however, appear to be directly linked to changes in mood state.

PRENATAL AMPHETAMINE EXPOSURE INCREASES THE NUMBER OF NEURONS POSITIVE FOR NITRIC OXIDE SYNTHASE (NOS) IN THE LATERAL/EXTRAMEDIAL TERMINAL NUCLEUS OF THE HAT. N. Nakano*, M. Nishizawa, and S. M. McTavish. Dep't of Biological Sciences, Univ. Southern California, Los Angeles CA 90089-2520, and Dept. Psychiatry and Behavioral Sciences, Univ. of Arkansas for Medical Sciences. Little Rock AR 72205.

A substantial amount of evidence suggests that at least some of the schizophrenia(s) are related to errors in fetal development. To test the fetal development hypothesis we have exposed rat pups in utero to dopaminergic agonists and have examined the behavior and neuroanatomy of these animals when they mature to young adulthood. NOS-positive cells in the lateral/nigro/tegmental nucleus (LDN) were of interest since these control phases of sleep which are deficient in schizophrenia. Twenty-three pregnant Wistar rats were injected on d 11-14 of pregnancy with d-amphetamine sulfate (5 mg/kg) or saline. Pups at 45-50 days of age were sacrificed by cardiac perfusion, after which cells of the LDN of selected horizontal sections were stained for NOS using NADPH and nitroblue tetrazolium. The number of NOS-positive cells observed in a field of 6.93 x 10⁴ μm² increased by 14% in amine treated with amphetamine (control = 42 ± 2 vs. 48 ± 6, p<0.01, treated = 40 ± 1 vs. 46 ± 1.3, n=10). The increase is greater in direction but is smaller than that reported for schizophrenia (Kane et al. Psychiat Res. Neuroimag (1995) 42: 37-49). The data suggest that certain aspects of the fetal development hypothesis of human schizophrenia can be demonstrated in an animal model. (Supported by MH and the Medcon Foundation).
AFFECTIVE DISORDERS

669.13
THE EFFECT OF ELECTROCONVULSIVE STIMULATION ON HIPPOCAMPAL LONG TERM POTENTIATION IN VIVO. C.A. Stewart, I.C. Reid and J.J. Whalley*. Dept. of Psychiatry, Univ. of Edinburgh, Edinburgh EH8 92Z, and *Dept. of Mental Health, Univ. of Aberdeen, Aberdeen, Scotland, UK.

Although electroconvulsive therapy (ECT) has been used as an effective treatment for severe depressive illness for 50 years, its mode of action, remains unknown. Apparent side effects of ECT include disruption to memory processes which have been documented in the medical literature. A recent study has suggested that electroconvulsive stimulation (ECS) in rats has profound effects on the induction of Long Term Potentiation (LTP) recorded from a hippocampal slice preparation (Avery et al, 1987, Brain Res.). Given the putative role of LTP in learning and memory, this finding provides a possible mechanism/explanation for the memory impairment associated with ECT.

In this study, the effects of repeated ECS on the induction and single ECS on the maintenance of LTP were examined in intact rats. Seizures were induced transcranially via ear-clip electrodes. Field potentials were recorded from the hilus of the dentate gyrus during low frequency stimulation of the perforant path in amphetamine animals. A series of 10 ECS spaced over 20 days impaired LTP induction as measured by the extracellular post synaptic potential (EPSP) slope function (control 24.6 ± 3.2%, ECS 11.1 ± 2.8%) and the population spike height (control 337 ± 75.2%, ECS 1.9 ± 5.0%). Examination of the absolute EPSP slope and population spike values pre-treatment indicated that the reduction in the amount of LTP obtained may have been due to the system being partially saturated. Preliminary experiments looking at the effect of a single ECS induced 25 minutes after tetanus suggest that the seizure reverses previously established LTP in an area above baseline population spike. Further experiments are in progress to determine the precise time course of these effects and their behavioural consequences.

Supported by the Stewart Sim Bequest, Royal College of Physicians, Edinburgh.

669.15
TREATMENT IMPLICATIONS OF BASELINE ANGER AND OF ANGER RESPONSE TO MCCP IN GENERALIZED ANXIETY DISORDER (GAD). M. Germain, M.D., A.W. Goddard, M.D.*, D.E. Sholomskas, Ph.D., G.R. Heninger, M.D., D.S. Charney, M.D., and S.W. Woods, M.D., Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06519.

In a group of 10 patients with GAD a marked anger response to the serotonergic agonist MCCP has been noted, which appears to show relative specificity to GAD. Baseline anger is predictive of net anger response (p=0.07) of net LTP change in Panic Attack Symptom Score (PASS) (p=0.03). No significant correlations were noted between baseline anxiety and any of these measures. After the MCCP challenges and baseline ratings conducted over 3 weeks off medication, a clinical trial of open-label buspirone was conducted on 8 of the patients. One patient dropped out during the first week due to side effects. Four patients were judged to be clinical responders and three non-responders by a research psychiatrist blind to the anger data. The responders had a mean net anger response to MCCP of 46±13mm (visual analog scale) compared to 0±1mm in the non-responders (p=0.002) and VAS baseline anger scores of 4.5±4.1mm vs 0±4.2 (p=0.12). No significant relationship was noted between net VAS anxiety response to MCCP and response to buspirone. Together these results suggest a relationship between baseline anger, net anger response to MCCP, and buspirone response in GAD. Although the neurobiological mechanisms underlying these relationships are not immediately apparent, these observations do suggest that baseline anger and/or anger response to MCCP may be useful predictors of clinical outcome, at least in GAD. Replication with larger samples is required.

669.16
ELECTROCONVULSIVE SHOCK (ECS) INDUCES LONG-TERM CHANGES IN REGIONAL BRAIN CYTOCHROME OXIDASE ACTIVITY. L.S. Nobrega*, S. Raymond and W.M. Burnham. Neuroimaging Research Section, Clarke Institute of Psychiatry, and Pharmacology Department, University of Toronto, Toronto, Ont., Canada.

Electroconvulsive therapy has clearly beneficial effects in severe depression. However, questions remain concerning its mechanisms of action and possible long-term deleterious effects on brain function. The present study used cytochrome oxidase (CO) histochemistry to examine long-term effects of ECS on regional brain metabolic activity. CO is a mitochondrial enzyme whose activity is tightly coupled to neuronal function (Wong-Riley, 77NS, 1989, f2, 94). Rats received a course of 8 ECS (once every 48 hr, 150 mA, .2 sec) and were sacrificed either 24 hr or 28 days after the last ECS or sham treatment. CO activity was quantitated in 99 different brain areas using calibrated densitometric standards. Twenty-four hour post ECS CO activity was generally elevated in ECS-treated brains, but in none of the regions examined did ECS vs. control differences reach statistical significance. In contrast, at 28 days CO activity in ECS brains was significantly increased in the bed n. of the stria terminals (+25%), lateral geniculate (+14%) and medial mammillary (+12%), dorsomedial (+20%) and ventromedial (+12%) hypothalami, ventromedial thalamus (+9%), intralaminar n. (+20%), mammillary n. (+14%) and pontine n. (+16%). The observed general tendency for increased CO activity after ECS is consistent with an absence of gross pathology. The observations at 28 days suggest that ECS induces progressive metabolic changes which seem to develop independently of additional stimulation. Finally, the fact that changes were seen predominantly in limbic structures suggest a possible involvement of these areas in the therapeutic effects of ECS.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
AFFECTIVE DISORDERS: SEROTONIN

Friday AM

SEROTONIN AND BETA-ADRENERGIC RECEPTOR BINDING IN FRONTAL CORTEX AND HIPPOCAMPUSS OF SUICIDE VICTIMS. C.A. STOCKMEIER*, Y. ZHANG, H.Y. MELTZER, J. OVERHOLSER, P.R. EINSSBERGER, P.A. THOMPSON AND L. KHATIAN. Dept. of Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

Abnormalities in serotonin (5HT) and beta-adrenergic receptor binding have been reported in frontal cortex of suicide victims. Brain samples were collected at autopsy from 12 violent suicide victims (gunshot or hang; male and 12 age and sex matched controls dying of natural or accidental causes or homicide. Psychological autopsy examinations revealed that 6 suicide victims had a history of depression. We used quantitative receptor autoradiography to measure the binding of [*]H*ketanserin, [*]H*8-OH-DPAT and [*]H*indolol to 5HT-1, 5HT-1A and beta-adrenergic receptors. In all samples, age was inversely correlated with 5HT-2 beta-adrenergic receptor binding. There were no significant differences between matched pairs of controls and all suicides or matched controls and suicides with a depressive illness for monoamine content or 5HT-1A, 5HT-2 or beta-adrenergic receptor binding in frontal cortex, or 5HT-1A or beta-adrenergic receptor binding in hippocampus.

Further studies with larger sample size and attention to effects of drug treatment and prior depression are needed. Supported by PHS Grants MH45488 and MH41684.

SEROTONIN 5-HT1B BUT NOT 5-HT1 RECEPTOR NUMBER IS INCREASED IN HIPPOCAMPUSS OF SUICIDE VICTIMS.


We have previously reported increased [*]I-LSD binding in the prefrontal cortex of suicide victims (Arango et al., Arch Gen Psychiatry, 1990). In order to determine whether this increase involves 5-HT1 or 5-HT2 receptors, we determined the proportion of 5-HT1 and 5-HT2 receptors in human cortex, choroid plexus and hippocampus.

We assayed 5-HT1 and 5-HT2 receptor binding using [*]I-LSD. Nonspecific binding was determined by 10M mianserine. 5-HT2 binding was blocked by 3.2nM cisapride in order to determine 5-HT1 binding. We performed 5 pairs of suicide victims and controls matched for age, sex, postmortem delay and season of death. All subjects had no history of toxicological screens. [*]I-LSD binding kinetics in both cortex and choroid plexus conformed to a single site model. Competition and saturation binding kinetics characterized the cortical site, while the choroid plexus binding site was the 5-HT1 receptor. No detectable 5-HT2 receptors were present in cortex. 5-HT1 receptor number (Bmax) was significantly greater in the hippocampus of suicide victims compared to controls (949.2 ± 28.7 ± 10.3) fmol/mg protein, p=0.043, 2-tailed Wilcoxon matched pairs). In contrast, 5-HT1 receptor number did not differ in suicides and controls.

Seasonal effects were significant for 5-HT1 receptor binding (p=0.003). The affinity (Kd) of both receptor populations did not differ between groups. This study indicates that the increase in [*]I-LSD binding in the cortex of suicide victims is unrelated to 5-HT1 receptors and provides further evidence for the biochemical specificity and regional localization of the alteration in serotonin function.

Supported by MH40210 and MH46745.


On some serotonin (5-HT) indices in brain, platelet and CF have been reported. Serotonergic abnormalities have been associated with depressive disorders and seasonal effects may be a component of the index of the magnitude and duration of episodes of depression. To determine the biochemical specificity of these effects, we studied the seasonal effects on the platelet 5-HT1 receptor and epinephrine-stimulated phosphoinositide (PI) hydrolysis in controls and patients with affective disorders.

Across all seasons, serotonin (0.2 mM) stimulated a 60.4 ± 5.1% increase in PI turnover in controls compared to 41.2 ± 5.1% in patients (p=0.008). Epinephrine (0.1 mM) associated increase in PI turnover was 34.3 ± 5.3% in controls compared to 17.6 ± 1.28% in patients (p=0.01). Seasonal effects were significant for 5-HT stimulated PI turnover in patients (p=0.01) and healthy controls (p=0.003). In patients, 5-HT stimulated PI turnover peaked during Summer (57.6 ± 4.2%) and was significantly higher than other seasons. In controls, there was a 24.8% increase from Spring to Summer. Seasonal effects differed in controls (p=0.005) because 5-HT stimulated PI responses were higher in Winter than Summer. No statistically significant seasonal effect was found for PI stimulated PI turnover in patients (p=0.075).

We conclude: (1) PI hydrolysis signal transduction is blunted in depressed patients; (2) Season selective differences in total PI turnover may be compared to EPI activation of PI hydrolytic (3) Platelet 5-HT1 and EPI stimulated PI hydrolysis in patients are more pronounced in Spring versus Summer. This finding may be related to the increased rate of depression and suicidal behavior in Spring. Supported by MH46745, MH48514 and MH46095.
670.7

EFFECT OF ALCOHOL-DEPENDENCE ON 5-HT A BINDING IN SUICIDE.

Serotonin alterations have been associated with suicidal behavior. Using quantitative autoradiography we sought to determine whether the postsynaptic 5-HT A binding is altered in the prefrontal cortex of alcohol-dependent suicide victims by studying nine Brodmann areas (8, 9, 11, 12, 24, 32, 45 and 47) of alcohol-dependent suicide victims and nonalcoholic controls with negative toxicological screens for other drugs. Alcohol-dependence was determined by conducting a psychological autopsy. Subjects were matched for postmortem delay, age and gender (N=4 pairs). In addition, a group of nonalcoholic suicide victims and matched controls were also studied (N=14 pairs). Slide-mounted coronal sections (20μm) from the right hemispheres were incubated with 2mM 3 H-DPAT as described previously (Arango et al., Soc. Neurosci. Abstr., 1991). 5-HT A receptor distribution across cortical layers was similar in all groups, forming five isodensity bands corresponding to layer I, layer II, outer layer III, layers III-IV, and layers V-VI. Layer II had the highest level of binding. The nonalcoholic suicide group had 20% more 5-HT A binding than the controls in area 46, but not in the other areas (p = 0.024). The alcoholic suicide group had elevated binding compared to controls in areas 24, 8, 9, 11, 12, 24 and 32. The binding in both suicide groups and controls was negatively correlated with postmortem delay. Females had greater 5-HT A binding than males. We conclude that 1. 5-HT A binding is increased in some brain regions of suicide victims; and 2. alcoholism and suicide was associated with a more widespread alteration in 5-HT A binding. This study provides further evidence for an association of serotonin and both alcoholism and suicide. Further studies should investigate other brain areas and other 5-HT receptors subtypes. (Supported by NIMH grants AA00904, MH46210 and MH64745.)

670.9

'N-CYANOPYRIMIDINE BINDING IS REDUCED IN SUICIDE VICTIMS.
B.W. Smith, V. Arango, M.L. Miller, M.D. Underwood and J.L. Mann, Laboratories of Neuropsychology, University of Pittsburgh, Pittsburgh, PA 15213.

We previously reported decreased 'H-paroxetine binding to the serotonin transporter in membrane homogenates from suicide victims compared to controls (Henriette et al., Soc. Neurosci. Abstr., 1991). Conflicting reports exist in the literature as to whether binding to the serotonin transporter on serotonin nerve terminals is reduced in brain cortical regions of suicide victims (Arango and Mann, Rev. Psychiatry, 1992). Using the selective radiolabeled 'H-cyanopyrimidine ('H-CNIMI), we sought to determine whether binding is altered in slide-mounted sections (20μm) from the prefrontal cortex (Brodmann areas 8, 9, 46, 45, 47, 11, 12, 24 and 32) of suicide victims compared to controls. All cases had negative toxicological screens. Subjects were matched for postmortem delay, age, gender, and where possible, race and season (N=13 pairs). Tissue was preincubated in 50mol Tris-HCl buffer with 130mM NaCl, 5mM KCl (pH 7.4, 30 min, 23°C) and then incubated (24 h, 4°C) in the same buffer containing 30pM PMSF and 0.4nM 'H-CNIMI. Nonspecific binding was determined by 10μM sertraline. After a 17 week exposure, films were developed and quantified by image analysis. The highest level of binding in both groups was localized to the outer layers of the anterior cingulate gyrus (Brodmann area 24), with considerably higher than the dorsal prefrontal cortex (areas 8 and 9). In area 46 (lateral aspect of the hemispheric) the suicide group had a 42% reduction in binding, compared to controls (Suicides: 5.36 fmol/mg tissue; Controls: 9.50 fmol/mg tissue, p<0.031). Binding did not differ between groups in any of the other areas examined. Binding was positively correlated with postmortem delay (p<0.05) but not with forensic storage, age or sex (p>0.05).

We conclude that 'H-CNIMI binding sites: 1) have a specific distribution in prefrontal cortex; 2) are reduced in a specific area of the prefrontal cortex in suicide victims; 3) correlate positively with postmortem delay; and 3) are not different in males and females. The reduction in binding is consistent with the hypothesis of a serotonergic deficiency associated with suicide. (MH40210 and MH64745.)

NEUROTOXICITY: BIOLOGICAL

671.1


Rats recovered from an acute bout of pyrithiamine induced thiamine deficiency (PTD) display learning and memory deficits and severe destruction of medial thalamus and mammillary bodies. Milder damage to limbic and cortical areas has been difficult to detect because of long recovery periods (3-6 months). In the present study male Sprague-Dawley rats were treated daily with a thiamine free diet and from 4-5 hrs after onset of seizures, levels of the EAAs dropped to 10-30% of basal levels in the dorsal prefrontal cortex (area 8 and 9). In area 46 (lateral aspect of the hemispheric) the suicide group had a 42% reduction in binding, compared to controls (Suicides: 5.36 fmol/mg tissue; Controls: 9.50 fmol/mg tissue, p<0.031). Binding did not differ between groups in any of the other areas examined. Binding was positively correlated with postmortem delay (p<0.05) but not with forensic storage, age or sex (p>0.05).

We conclude that 'H-CNIMI binding sites: 1) have a specific distribution in prefrontal cortex; 2) are reduced in a specific area of the prefrontal cortex in suicide victims; 3) correlate positively with postmortem delay; and 3) are not different in males and females. The reduction in binding is consistent with the hypothesis of a serotonergic deficiency associated with suicide. (MH40210 and MH64745.)

671.2


An excitotoxic basis for thiamine deficiency encephalopathy has been suggested by the demonstration that MK-801 prevents thalamic lesions in pyrithiamine treated (PTD) rats (Langlais & Mair, J. Neurosci., 1992). The current study measured extracellular fluid levels of the excitatory amino acids (EAA's) before and during the onset of PTD induced seizures and pathologic lesions. Male Spraque-Dawley rats were treated with daily pyrithiamine (0.25 mg/kg, i.p.) and then injected with 3H-glutamate and 3H-aspartate 30 min prior to seizures. Basal levels were obtained at a pre-lesion stage (9-11 days of PTD treatment). No significant change from basal levels were observed in the EAAs. In dialysates collected 12 hrs after onset of seizures, levels of the EAAs fell to 10-30% of basal levels. Neuronal loss within thalamus containing the probe was noticeably less severe than on the undialyzed side, presumably due to the removal of EAAs. Supported by NIH grant NS2948101 and VA Merit Program Award to P.JL.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
671.3 EFFECTS OF NICOTINE ON RETINAL DEVELOPMENT.
S. He, T-X. Jiang, C-M. Chuong, D.R. Hinton*
Department of Pathology, USC School of Medicine, Los Angeles CA 90033
Prenatal exposure of rats to high doses of nicotine via maternal infusion can impair nervous system development in association with decreased fetal viability and growth. Persistent alterations in certain parameters of neural cell development also occur at lower doses of nicotine which do not impair intranatal growth. The retina serves as an excellent model for developing nervous tissue because of its rapid and well-documented developmental sequence.
Timed pregnant rats were implanted on embryonic day E15 with minipumps containing nicotine bitartrate or sodium bitarate (control). Serum levels of nicotine in experimental groups averaged 200 μg/ml. Feasures were sacrificed at days E18 and E21. Eyes were dissected, fixed in 4% paraformaldehyde and embedded in glycol for morphological examination. Animals from experimental groups were killed on day 1 (E15) and day 2 (E16) after birth to examine changes in retinal parameters. Changes were noted at E18 and prominent at E21. Ganglion cell number did not appear to be affected.
 Cultures of newborn rat retina were examined in monolayer and dissociated. The number of cells extending neurites was similar, nicotine treatment at 100 μg/ml and above resulted in significant shortening of neurites. These experiments suggest that nicotine is acting on retinal tissues by affecting differentiated neuronal functions such as neurite outgrowth.

671.4 BRAIN EDEMA IN HEPATIC ENCEPHALOPATHY (HE) AND HYPERAMMONEMIA. W. Hilger and J.R. Olson*
Brain edema in HE has been shown to be due to brain edema while potassium loss represents a mechanism of water homeostasis. Supported by NS 23218 and Kettering Medical Centre, Medical Education.

671.5 CENTRAL EFFECTS OF T-2 TOXIN, A TRICHOTHECENE MYCOTOXIN. J. Wang, R. Wilson* and D.W. Fitzpatrick.
Departments of Foods and Nutrition and Psychology, University of Minnesota, Minneapolis MB 837 202 Canada.
The effect of T-2 toxin on the brain was examined. To examine the dose-dependent effect of T-2 on neurotransmitter, rats were orally dosed with T-2 QO.1, 1.0 or 2.5 mg/kg BW; killed 6 and 10 hours post toxicity; and brain nuclei were analyzed. T-2 treatment increased 5-hydroxytryptamine (5HT) and dihydroxyphenylacetic acid (DOPAC) were observed. Few treatment differences were observed, with 0.1 mg/kg increasing 5HT and DOPAC, 2% of the normal level, whereas the control group received no alcohol. The alcohol group also received ethanol (400 mg/dl) along with the normal serum, whereas the control group received no alcohol. The alcohol control group had identical results and revealed that Purkinje cells expressed NGFR, with the greatest expression at this age present in more posterior cerebellar lobules. Purkinje cells in more mature areas of the cerebellum displayed prominent NGFR in their dendritic fields, whereas less developed cells had NGFR only in the proliferative zone; no NGFR was evident in the presumptive zone.

671.6 ALCOHOL REDUCES NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY IN NEONATAL RAT CEREBELLUM. D. Doughman, J.R. West, CR. Goodlett and VJ. Piantadosi. Dept. of Anatomy, Univ. of Iowa Medical School, Iowa City, IA 52242.
Nerve growth factor receptor (NGFR) is expressed during a restricted time (postnatal days 6-20) in the rat cerebellum. NGFR, a potent neurotrophic agent, may play a critical role in the development of cerebellar Purkinje cells. Alcohol exposure during development depletes cerebellar Purkinje cells in the rat, with the most severe depletion occurring when alcohol is administered at a time which approximates NGFR expression on Purkinje cells. This raises the possibility that alcohol depletes Purkinje cells by disrupting the interaction between these cells and neurotrophic factors, such as NGF. Since NGF activity is mediated via NGFR, this study examined whether alcohol alters the expression of NGFR in the cerebellum. Rat pups (4 days) were divided into 3 groups. The alcohol group was artificially nursed and fed ethanol via a gavage tube from days 4-10. The gavage control group was artificially reared and fed normal diet from days 4-10. The control group was reared normally and received no alcohol. At day 10, all animals were perfused and cerebellar sections were immunostained for NGFR with 192-14g antibody. The two control groups had identical results and revealed that Purkinje cells expressed NGFR, with the greatest expression at this age present in more posterior cerebellar lobules. Purkinje cells in more mature areas of the cerebellum displayed prominent NGFR in their dendritic fields, whereas less developed cells had NGFR only on their soma and dendrites. The external granule layer also expressed NGFR, but only in the proliferative zone; no NGFR was evident in the presumptive zone.

671.7 ALCOHOL INHIBITS PROLIFERATION OF NEURONAL-LIKE CELLS (PHROCHROMOCYTOMA, PC12) IN CULTURE. J. Luo, C.R. Goodlett, J.R. West and VJ. Piantadosi. Dept. of Anatomy, Univ. of Iowa Medical School, Iowa City, IA 52242.
Animal studies have shown that fetal alcohol exposure depletes selected populations of neuronal cells in the CNS. Although killing of cells by alcohol has often been suggested as a potential mechanism for this loss, alcohol can also reduce cell numbers by inhibiting (slowing) cell proliferation. This latter possibility was the focus of this study. Because it is difficult to determine mechanisms of alcohol action in animals, an in vitro neuronal cell model (PC12) was used, incorporating a novel approach (synchronized cultures of PC12 cells) to examine alcohol's effect on cell proliferation.
PC12 cells were synchronized by feeding the cells with media (RPMI) containing low serum (0.08% fetal calf serum, FCS, 0.17% horse serum, HS). This low serum treatment greatly reduced the number of cells and the cells arrested in the G1 phase of the cell cycle. Three days later, growth-arrested cells were fed normal serum (5% FCS, 10% HS) which stimulated synchronized growth of the cells (i.e., the vast majority of cells were at the same point in the cell cycle and progressed synchronously through the cycle). The alcohol group also received ethanol (400 μg/ml) along with the normal serum, whereas the control group received no alcohol. The alcohol concentration range tested was 200 μg/ml. After 48 hours, the cultures were examined and the number of cells extending neurites was similar, nicotine treatment at 100 μg/ml and above resulted in significant shortening of neurites. These experiments suggest that nicotine is acting on retinal tissues by affecting differentiated neuronal functions such as neurite outgrowth.

671.8 EFFECT OF NICOTINE ON NEURAL CREST CELL MOTILITY AND NEURITE ELONGATION IN VITRO. T.J. Jiang, C.K. He, D.R. Hinton and C.M. Chuong*. Dept. Pathology, Univ. Southern California, Los Angeles, CA 90033.
It has been shown that prenatal exposure to nicotine influences the development of the nervous system. To analyze the specific effects of nicotine and the mechanism of nicotine (10 ng - 10 μg/ml) on neural development, we used primary neural crest cell cultures and dorsal root ganglion explants for the evaluation of the effect of nicotine. In trunk neural crest explant cultures, crest cell were plump, spindle shaped, with long axis of cells randomly arranged, and the advance margin was very uneven, suggesting active cell motility. In contrast, in the presence of nicotine, crest cells were polygonal in shape, arranged regularly in palisades and the advance margin was even, suggesting lower cell motility. Time lapse video confirmed the difference in cell motility. In nerve root ganglion explant cultures, nicotine caused a decrease in neurite growth (by stage 3). Nicotine showed significant inhibition of neurite length at concentration of 10 ng/ml. At 10 μg/ml, there was complete inhibition. The number of dying cells and the degree of lysis of explant cultures were increased in the nicotine treated explants. There is no apparent difference in Immunofluorescent staining patterns of adhesion molecules H-CAM and NG-CAM. This suggests that nicotine does not specifically inhibit ganglion cell growth and neurite elongation. The results suggest that nicotine has inhibitory effect on cell motility and neurite elongation in vitro. We are currently studying the mechanism of these effects and evaluating the effect of nicotine on neural development in vivo.
671.9
NEUROTOXICITY: BIOLOGICAL


Several investigators have reported neuronalanatomical changes in both rat and primates after exposure to either THC or marijuana (MJ) smoke. Our studies were designed to use unbiased stereologic methods to assess the putative changes in primate brain after long-term exposure to MJ smoke. Fifteen male rhesus monkeys were divided into three groups and exposed to either placebo smoke (P), moderate MJ smoke (M), or high MJ smoke (H). These subjects were implanted with an array of intracerebral glass electrodes before MJ exposure. Three unseeded control monkeys were also included for study. After completion of the behavioral and electrophysiologic testing, all animals were sacrificed by per cardiac infusion with aldehyde fixative. This surgical study was based on the high concentration of cannabinoid receptors in its molecular layer. The cerebella were sagittally sectioned, weighed, and blocked for light and electron microscopy. One concentration of cannabinoid receptors in its molecular layer. The cerebella were

671.11
TETANUS TOXIN ENTERS NEURONS THROUGH ACIDIC ENDOSESOMES. L.C. Williams*, W. Y. Clarke, S.C. Fitzgerald and E. A. Neale. Laboratory of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Tetanus toxin (TnTx) acts preferentially on the inhibitory synapses to block neurotransmitter release. In murine spinal cord cell cultures, TnTx inhibits taurine-dependent evoked release of glycine. To test whether TnTx enters neuronal cytoplasm through acidic endosomes, toxin action was assayed in cultures pretreated with monensin to neutralize acidic compartments. Monensin (0.5 μM) completely blocked the effect of TnTx, presumably by interfering with its movement from endosomes into the cytoplasm. Monensin effects were reversible and the drug had no effect on toxin binding. If an acid environment were required for toxin translocation across endosomal membranes, lowering the external pH should allow the entry of toxin directly through the cell membrane, by-passing the endosome. Cultures were exposed to TnTx at 4°C to achieve toxin binding without endocytosis, and then were pulsed for 6 min in the cold with medium at pH 7.25 or pH 4.9. Monensin was added and the cultures warmed. Inhibition of evoked glycine release was observed in toxin-exposed cultures in the presence of monensin when cultures were pulsed at pH 4.9. However, there was no inhibition of evoked glycine release in cultures pulsed at pH 7.25. These findings indicate that TnTx requires an acid environment to penetrate cellular membranes. 3-γ-D-Methylpropylamino (DAMP) accumulates in acidic compartments and can be visualized by immunohistochemistry as punctate staining in neurons and glia in spinal cord cultures. Monensin reduced DAMP accumulation in neurons and glia. If, in the presence of acid, TnTx forms pores in membranes, it could disrupt the pH gradient in endosomes and likewise prevent DAMP accumulation. In cultures exposed to toxin, DAMP immunoreactivity was reduced in neurons although immunoreactive glia was similar to controls. These data suggest that TnTx enters the neuronal cytoplasm through an endosome pathway.

671.10
BOTULINUM NEUROTOXIN SEROTYPES A, B AND E EXHIBIT PROTEOLYTIC ACTIVITY. B. R. DasGupta* and W. Tepp. Food Research Institute, Madison, WI 53706.

Botulism toxin [NT] serotypes A, B and E are encoded by genes found either in the chromosome, plasmid or a phage hosted by Clostridium botulinum. The -150 kDa single chain NT after posttranslational proteolytic processing (nicking) is a dithiol protein made of -50 kDa L- and -100 kDa H chains that correspond to the N- and C-terminal segments of the parent protein; -S-S- bonds (1) link L and H chains. The NT binds via H chain to the presynaptic membrane at the neuromuscular junctions. L chain after entering the secretory cells inhibits neurotransmitter release by an activity presumed to be enzymatic, the substrate of which is unknown. We report that the NT types A, B and E nick and cleave at pH 5-8 show self-digestion—evidence is time dependent discrete bands in SDS-PAGE. The self-digestion is promoted following nicking and DT reduction. The dichain type E NT cleaved actin (rabbit muscle) into discrete fragments. The L chain of type E NT, separated from the H chain, digested actin more effectively than the parent NT. These new data indicate, consistent with earlier reports (Biochem J 7, 1193, '89; J. Physiol. (Paris) 84, 220, '90; Soc. for Neuroscience Abst. 10, 609, '90; 17, 1526, '91; PAFER 3, 6, 427, '92; J. Neurochem. 57, 14, 47, '91) that the NT is a protease and suggest that the NT types C and D represent phage encoded proteases. The peptide bonds cleaved by the proteolytic activities of the three NT types are under study. Funded by NS17742.

671.12
CYTOXIC ACTION OF PALYTOXIN IN AORTIC SMOOTH MUSCLE CELLS IN CULTURE. R. E. Sheridan*, B. E. Doxzon and S. S. Deshpande. Neurotoxicology Branch, Pathophysiology Division, USAMRICD, Aberdeen Proving Ground, Maryland 21005-5425, USA.

Palytoxin (PTX) produced by gastropod species Palythoa is a potent marine toxin which depletes neurons, skeletal, smooth and cardiac muscles. In addition to direct excitotoxicity, PTX induces intense vacuolization and hemerhage which contribute to lethality (Toxicon 1974). A7r5 cells derived from fetal rat aorta have many characteristics of smooth muscle (Exp Cell Res. 253, 34, 1976). We have used the A7r5 cell line to further delineate the mechanism of toxic action of PTX. A7r5 cells were grown in 35 mm dishes to confluence (4-10 days) in DMEM supplemented with 5% fetal bovine serum under standard conditions. Exposure of the PTX (10 μM) for 15 min at room temperature followed by wash and incubation in culture medium at 37°C (≤ 30 min) led to swelling, clumping of cytoplasm, vacuolation and shrinking of heterochromatin. These cells were nonviable as confirmed by exclusion of trypan blue and release of LDH. Concentration–response determination for PTX (3 X 107 to 4 X 107 M) using the vital dye assay gave an EC50 value of 7.1 X 107 M. PTX-induced cytotoxicity could not be reversed by washing. Prior treatment of cells with calcium (10 μM) or vanadate (10 μM) for 30 min reduced the cytotoxic effects produced by PTX. Whole-cell patch clamp recording from single A7r5 cells showed that PTX (0.1 X 107 to 10 μM) induced depolarization of cells that activated non-voltage controlled Na+ and Ca2+ (Wang et al., Soc. Neuroscience, 1989). The precise mechanism responsible for the cytotoxic effects of PTX on A7r5 cells is presently unclear, but seems to involve Na+ and Ca2+ overload. In the light of the involvement of smooth muscle in PTX poisoning, A7r5 cells could serve as a useful model to test specific drugs for protection against PTX toxicity.
672.1


Monosialoglacosidosides (GM1, GD1a, GD1b, GQ1b) are a group of gangliosides which have been found to prevent neuronal injury. These compounds are used to treat various neurological disorders such as Alzheimer's disease. The present study examined the effects of these compounds on the brain content of glycosphingolipids in an animal model of neuronal injury. The results indicate that oral administration of these compounds significantly increased the levels of GM1 and other gangliosides in the brain, indicating their potential therapeutic benefits in the treatment of neurological disorders.

672.2


Several amphetamine analogues, when administered in high dose regimens, have been shown to cause long-lasting depletion of CNS serotonin (5-HT) which can be attenuated pharmacologically. Several laboratories have shown that MK-801 (a non-competitive NMDA receptor antagonist) protects against this toxicity. The present study was to determine whether MK-801 protects against 5-HT toxicity by increasing the temperature. MK-801 was administered to rats which were then housed at different temperatures. The results indicate that MK-801 protects against 5-HT toxicity by increasing the temperature, which suggests a possible role for temperature increase in the neuroprotective effects of MK-801.

672.3


Intrastriatal injections of quinolinic acid (QA) cause increased release of glutamate from glial cells. The present study examined the effects of excitotoxic exposure on the release of glutamate from glia. The results indicate that exposure to QA increases the release of glutamate from glial cells, which may contribute to the neurotoxic effects of QA.

672.4

NIACINAMIDE BLOCKS THE EFFECTS OF 3-ACETYLGLYCINE IN CEREBELLAR GRANULE NEURONS IN VITRO. M. Weller, A. M. Marini and S. M. Paul*. Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892.

3-Acetylpyridine (3AP) is a potent experimental neurotoxin which preferentially damages the inferior olivary nucleus when administered in vivo. The present study examined the effects of niacinamide on 3AP-induced toxicity in vitro. The results indicate that niacinamide protects against 3AP-induced toxicity, suggesting a potential therapeutic role for niacinamide in the treatment of 3AP-induced neurotoxicity.

672.5


Intrastriatal injections of quinolinic acid (QA) cause increased release of glutamate from glial cells and neuronal loss. The present study examined the effects of QA on the blood-brain barrier. The results indicate that QA causes breakdown of the blood-brain barrier, which may contribute to the neurotoxic effects of QA.

672.6


Intrastriatal injections of quinolinic acid (QA) cause increased locomotor and seizure activity. The present study examined the effects of QA on spatial learning abilities. The results indicate that QA causes spatial learning deficits, which may contribute to the neurotoxic effects of QA.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 18, 1992
Neurotoxicity: Excitotoxins

672.7 N-Methyl-D-aspartate receptor-mediated neuroprotection in cerebellar granule cells requires new RNA and protein synthesis. A. M. Martin, P. Damshofer-Williams, and S. M. Paul, CBN, NYC. Bethesda, MD 20895.

Cultured cerebellar granule cells are glutamatergic neurons which express all of the glutamate receptor subtypes. Previous studies have shown that cultured cerebellar granule cells are sensitive to the neurotoxic effects of the excitocin glutamate and the chemical neurotoxin, 1-methyl-4-phenylpyridinum (MPP+). Although the toxicity of glutamate and MPP+ are time- and concentration-dependent in cultured cerebellar granule cells, the mechanisms of toxicity are different. Whereas glutamate mediates its toxicity by the activation of N-methyl-D-aspartate (NMDA) receptors, MPP+ kills neurons by intracellular uptake into vulnerable neurons. Paradoxically, preconditioning of cultured granule cell neurons with subtoxic concentrations of MPP+ or glutamate markedly antagonizes the neurotoxicity from subsequent exposure to toxic concentrations of either MPP+ or glutamate. The neuroprotective effects of MPP+ and glutamate against MPP+ toxicity are observed at concentrations as low as 1 μM, blocked by specific NMDA receptor antagonists and require at least 30 min to fully develop. Preconditioning of the neurons to subtoxic concentrations of MPP+ also resulted in significant protection against toxic glutamate concentrations (50-100 μM). Moreover, MPP+-receptor mediated neuroprotection is prevented by the RNA synthesis inhibitor, actinomycin D, or the protein synthesis inhibitor cycloheximide. Thus, activation of NMDA receptors by glutamate results in either neuroprotection or neurodegeneration, depending on the glutamate concentration and apparent degree of receptor stimulation. MPP+ receptor-mediated neuroprotection in these neurons requires new RNA and protein synthesis and appears to be mediated by the expression of a neuroprotective protein. Taken together, these data demonstrate the presence of an active MDMA receptor-mediated and transcriptionally-directed neuroprotective mechanism in cerebellar granule cells.


bFGF stimulates proliferation and subsequent differentiation of a variety of mesenchymal cell types. In vitro, this factor demonstrates neurotrophic activity. In the central nervous system bFGF, seems to be constitutively expressed, and in the rat hippocampus in situ hybridization reveals low levels of bFGF expression in field CA2. These findings support the notion that this potent neurotrophic factor may be involved in CNS development and maintenance and that it could act as a survival factor in the adult CNS. To test this hypothesis, we have studied the regulation of the bFGF gene following neuronal hyperactivity induced by kainic acid (KA). In vitro and in vivo. In vivo models include intraperentinal and intra-amygdala injections of KA while the in vitro experiments were conducted with hippocampal slices. bFGF mRNA expression was monitored using in situ hybridization and reverse transcriptase coupled PCR. We have observed that three hours following seizure activity, bFGF mRNA expression was increased in fields CA1 and CA2 when compared to control animals and that this upregulation was transient.


Kainic acid (KA) while the in vitro experiments were conducted with hippocampal preparations showed neuronal damage in the left CA3-4. Preexposure of KA hippocampus to kainate neurotoxicity in the rat hippocampus.


Recently complement receptor, activated complement products, and complement mRNAs were demonstrated to be expressed in normal human and rat brains. We also showed up-regulation of complement mRNAs in AD brain in association with AD type lesions, and in experimental rat brain lesions. In the present study, rat brains were isolated, post-fixed in perfusion, and processed in situ hybridization analysis. The major increase of C1qB mRNA was observed in the dentate gyrus layer of the hippocampus 48 hours after KA induced seizure activity. Northern blot hybridization analysis of total RNA isolated from microdissected hippocampus showed elevation of C1qB mRNA signal. In situ hybridization analysis of the major increase of C1qB mRNA was observed in the dentate gyrus layer of the hippocampus 48 hours after KA induced seizure activity. Northern blot hybridization analysis of total RNA isolated from microdissected hippocampus showed elevation of C1qB mRNA signal. In situ hybridization analysis of the major increase of C1qB mRNA was observed in the dentate gyrus layer of the hippocampus 48 hours after KA induced seizure activity.

672.10 ZINC ENHANCES KAINATE NEUROTOXICITY IN THE RAT HIPPOCAMPUS. K. Shiraishi*, S. Nakazawa, H. Ito*.

Department of Neurosurgery*, and Anatomy, Nippon Medical School, Tokyo, Japan.

Zinc is one of the N-methyl-D-aspartate (NMDA) antagonists and has recently been known to potentiate non-NMDA currents in Purkinje cells expressing mRNA for an excitocin receptor clone (GluR3). Histochemical studies proved that the zinc distribution was localized in the hippocampus, CA3-4, where kainate binds specifically. We designed to examine whether the neuropharmacological effects of zinc could exist and find areas where they would develop in the rat brain. In the present study, zinc was administered into the intrahippocampal injections of colchicine. By in situ hybridization C1qB mRNA was increased in colchicine lesioned hippocampus (ipsilateral to the lesion). The highest increase of the signal was observed 10 day post lesion distribution mainly in areas adjacent to granule neuron layer, subiculum and outer molecular layer of CA1. These results suggest possible role of zinc in the inhibition in association with spinal cord function. (Supported by AG 00993-10).


Seizures induced by kainic acid (KA; 12 mg/kg ip) cause extensive neuropathological damage in the piriform cortex. To measure oxidative stress in brain during KA-induced excitotoxicity-rodent model, H2O2, glutathione peroxidase, catalase, glutathione, and protein antioxidants were measured. KA induced massive CA1 neuronal loss in CA1-CA3 regions whereas the underlying strata showed only moderate to low neuronal damage. Paradoxically, preincubation of cultured granule cell neurons with subtoxic concentrations of NMDA or glutamate markedly antagonizes the neurotoxicity from subsequent exposure to toxic concentrations of either NMDA or glutamate. The neuroprotective effects of NMDA and glutamate against MPP+ toxicity are observed at concentrations as low as 1 μM, blocked by specific NMDA receptor antagonists and require at least 30 min to fully develop. Preconditioning of the neurons to subtoxic concentrations of MPP+ also resulted in significant protection against toxic glutamate concentrations (50-100 μM). Moreover, MPP+-receptor mediated neuroprotection in these neurons requires new RNA and protein synthesis and appears to be mediated by the expression of a neuroprotective protein. Taken together, these data demonstrate the presence of an active NMDA receptor-mediated and transcriptionally-directed neuroprotective mechanism in cerebellar granule cells.
673.1 IMMUNOCYTOCHEMICAL LOCALIZATION OF CATALASE IN RAT BRAIN.
S. Monno and F. Magnusson* Lab. of Neurochemistry, Univ. of Connecticut, New Haven, CT 06511.
Catalase is a peroxisomal enzyme whose physiological functions are not well understood. We have established an immunocytochemical localization of catalase in rat brain using a polyclonal antibody raised against a recombinant rat catalase. The antibody showed a high affinity for catalase and was able to detect the enzyme at concentrations as low as 0.1 ng/ml. Immunohistochemical staining with the antibody revealed a specific and intense positivity in the rat brain. The antibody stained primarily the white matter, with a lower signal intensity in the gray matter. The staining was dense in the nuclei of the corpus callosum, the internal capsule, and the thalamus, and was also present in the basal ganglia and the cerebellum. The staining pattern was consistent with the presence of catalase in peroxisomes, which are known to be present in brain. These findings suggest a possible role for catalase in the detoxification of hydrogen peroxide in the brain.

673.3 GLUTAMATE HYPOTHESIS FOR TARDIVE DYSKINESIA. P.E. Andreini* and L.M. Gunne Dept. of Psychiatry, Umeå University, S-901 87 Umeå, Sweden.
Tardive dyskinesia (TD) is a well-known side effect of prolonged antipsychotic treatment. Although the exact mechanism of TD is not fully understood, the glutamate hypothesis has gained significant attention. This hypothesis suggests that chronic exposure to antipsychotics leads to an alteration in the glutamatergic neurotransmission. In a recent study, we investigated the role of glutamate in the development of TD in Cebus monkeys. Monkeys were treated with dopamine-depleting agents and then exposed to chronic antipsychotic treatment. Our results showed a significant increase in the concentration of glutamate in the striatum, a region known to be involved in motor control. These findings support the glutamate hypothesis and suggest that glutamate dysregulation may contribute to the development of TD.

Parkinson’s disease is a neurodegenerative disorder characterized by motor symptoms such as tremor, bradykinesia, and rigidity. Levodopa is the standard treatment for Parkinson’s disease, but it can also cause adverse effects, including dyskinesias and levodopa-induced fluctuations. In a recent study, we investigated the toxic effects of levodopa on cultured cells. We found that levodopa induces a time-dependent decrease in cell viability, suggesting a cytotoxic effect. This effect was more pronounced in dopaminergic neurons, which are particularly sensitive to levodopa toxicity. These findings highlight the need for further investigation into the mechanisms underlying levodopa toxicity and the development of strategies to mitigate these effects.
763.5
2-AMINO SUBSTITUTED MPTP DEPLETES BRAIN SEROTONIN AND NORPHEMINE Without AFFECTING DOPAMINE IN C57BL/6 MICE. A. M. Andrews, N. A. Garic*, and D. L. Murphy. Lab of Comparative Neurology, National Institute of Mental Health, Bethesda, MD 20892.

Both 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-(2-hydroxyphenyl)-1,2,3,6-tetrahydropyridine (2′-OH-MPTP) are known dopaminergic neurotoxins. Experiments with both 2′-substituted derivative of MPTP, namely 1-methyl-4-(2′-aminophenyl)-1,2,3,6-tetrahydropyridine (2′-NH2-MPTP), produced considerably different results following systemic administration (4 x 10 mg/kg i.p.) to C57BL/6 mice. Amino neurotransmitters and their metabolites were quantitated by HPLC-EC in mice sacrificed 1 and 3 weeks post-treatment. While 2′-CH3-MPTP (2 x 10 mg/kg) caused dopamine (DA) and its primary metabolites to be decreased substantially (~80%) in key brain regions such as striatum, 2′-NH2-MPTP produced marked depletions (~70%) in serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and norpamine (NE) in frontal cortex and hippocampus. Smaller decreases in 5-HT, 5-HIAA, and NE were seen in brain stem and striatum as well. 2′-NH2-MPTP had no effect on dopaminergic neurochemistry. Our current studies are investigating whether 2′-NH2-MPTP or a metabolite has a different specificity than other MPTP analogs for amineergic transport system.

763.7

Male Sprague-Dawley rats were administered DFEN (0-12 mg/kg, twice daily, per os) for 4 days and were sacrificed at either 24, 48, 66 hr, or 2-3 weeks after the last injection. Higher doses of DFEN produced a marked and long-lasting depletion of brain 5-HT content and uptake sites (by pargyline preâmbition). SHT immunohistochemistry showed the previously-described swollen 5HT-reactive axons at short (<1 mm) tracts in frontal cortex. As a measure of possible degenerative changes, we also studied responses of glial cells in the vicinity of swollen SHT-reactive processes. No evidence for an astrocytic reaction was obtained in either sections or Western blots of tissue extracts immunostained for glial fibrillary acidic protein (GFAP). In double-stained sections, there was no GFAP reaction even adjacent to SHT-immunoreactive swollen processes. Likewise, sections stained for reactive microglia (Griffonia simplicifolia) showed no change in the number or intensity of microglia at any time after DFEN. Supported by a grant from IRIS (Servier).

763.9

Sonam is an organophosphorus compound, an irreversible inhibitor of cholinesterase (ChE), and a potential nerve agent. Pridostigmine (pyrido) is a carbamate, a reversible inhibitor of ChE that has been used in recent years for choice of protection against nerve agent exposure. Last year at this meeting we reported changes in visual function following soman exposure. These studies were done to evaluate the protection to the visual system when pyridine pretreatment is used. Adult male cats were prepared and maintained with halothane anesthesia and maintained throughout the experiment with urethane. Following base line visual evoked potential (VEP) collection, enough pyrido was given to inhibit 30-80% of blood ChE. Soman (4 μg/kg) was then given. Following soman, there were no changes in VEP. Inhibition of blood ChE and 80-90% VEP reduction with no recovery over the next 24 hours. Following recovery of blood ChE and pyrido did not alter the VEP. Following soman, the recovery of the VEP was over 24% compared to nonsonam blood pyrido and blood ChE returned to about 60% within several hours. Even with less pyrido pretreatment, significant recovery occurs within several hours.

763.10

Toxicity of organophosphate compounds (OPs) has long been associated with their ability to inhibit acetylcholinesterase (AChE). Recently research has surfaced indicating direct interaction of OPs with mucaracine acetylcholinesterase receptors in the CNS. We investigated the sensitivity of mucaracine receptor binding to N-methylatol (NMA) and diazoxan (DI) to competition with a series of OPs in washed homogenates of frontal cortex from adult male Long Evans rats. The OPs' IC50s for competition with radiolabeled ligands were compared to the IC50s for the inhibition of AChE activity in the in vitro assay. Diazoxan (DI) and methyl-dioxolane (CD) blocked the receptors with IC50s in the range of 10-30 μM. The ability of IMA to block the receptors was dependent upon the concentration of IMA. The results demonstrate that diazoxan and methyl-dioxolane are more effective in blocking the receptors than IMA. The results demonstrate that diazoxan and methyl-dioxolane are more effective in blocking the receptors than IMA. The results demonstrate that diazoxan and methyl-dioxolane are more effective in blocking the receptors than IMA.
673.11 CHANGES IN CELLULAR CALCIUM HOMEOSTASIS AND TOXICITY OF 2,3-DICHLOROPHENYL DIMETHYLSULFOXIDE IN RAT CEREBELLAR GRANULE AND PONTINE NUCLEI. R.P. Kovesdai, O. Shin, H.A. Tilton* and G.J. Hardy. Neurotoxicology Division, NIEHS, Research Triangle Park, NC 27711.

Some polybrominated biphenyls (PCB) affect locomotor activity and brain dopamine function in laboratory animals. The exact mechanism of these effects, however, is not known. The uptake and release are dependent on the maintenance of normal calcium homeostasis of the nerve cell, we have studied the effects of a potentially neurotoxic PCB congener, 2,2'-dichlorophenyl (DCBP), on Ca++. In vivo measurements were performed in cerebellar granule cell cultures. Perturbations in calcium homeostasis have also been closely associated with cell death caused by a variety of toxicants. Free Ca++ measurements (Fura-2 fluorescence) and cytotoxicity (LD50) benchmarks were performed in cerebellar granule cells. Perturbations in calcium homeostasis have also been closely associated with cell death caused by a variety of toxicants. Free Ca++ measurements (Fura-2 fluorescence) and cytotoxicity (LD50) benchmarks were performed in cerebellar granule cells. Perturbations in calcium homeostasis have also been closely associated with cell death caused by a variety of toxicants. Free Ca++ measurements (Fura-2 fluorescence) and cytotoxicity (LD50) benchmarks were performed in cerebellar granule cells.

673.12 EFFECTS OF NEONATAL MONOSODIUM GLUTAMATE ON PEPTITE mRNA EXPRESSION IN RATTLEBRIDGE ENDOCRINE AND VISUAL CNS STRUCTURES. S.E. Bach*, W.S. Young, III, M. Palkovits. Lab of Cell Biology, NIH, Bethesda, Md. 20892.

Since we found that electrolytic arcuate-premammillary lesions moderate levels of mRNA for galanin (GAL), vasopressin (VP), angiotensin (AI) and cholecystokinin (CCK) in hypothalamic magnocellular neurons (Bach et al., 17:1186), we investigated whether arcuate lesions caused by neonatal monosodium glutamate (nMSG) produce similar effects. In addition, because MSG causes optic nerve atrophy, we measured CCK and substance P (SP) mRNA levels in the visual system structures, the dorsal nucleus of the lateral geniculate (DLG) and superficial layers of the superior colliculus (SC). Ratt pups received 4 mg/kg MSG, or 0.9% saline, s.c., on days 0, 2, 4, 6 and 8, and were sacrificed on day 35. Peptide mRNA levels were measured by hybridization histochemistry using 125I-digoxigenin labeled cDNA probes. Arcuate levels of mRNAs for neuropeptide Y (NPY), GAL, and neuropeptide B were depleted by nMSG, and levels for proopiomelanocortin (POMC) were reduced to 30% of control (p<.005). (Similar results using immunohistochemistry for NPY, GAL, and POMC gene products were reported by Meister et al., Exp. Brain Res. 76:343). GAL mRNA was double in the paraventricular nucleus (p<.0005) and increased by 17% in the supraoptic nucleus (p<.05) by nMSG. Magnocellular CCK, VP, and AI mRNA levels were not affected. In the SCs, while the density of label was comparable, the area was smaller after nMSG, so that the total label was reduced to 30% of control for SP (p<.001) and to 28% for CCK (p<.001). In the DLO, nMSG increased the density of CCK mRNA by 21% (p<.005), but because the area was reduced, the total label was reduced to 66% of control (p<.001). Thus, in addition to alterations in the cytoskeletal intermediate filament, a new set of neurotoxic agents was affected peptide gene expression in hypothalamic nuclei, the nMSG-induced damage to optic fibers is not often seen in rats because the dose curves for lethality are very steep. Though other animal models are used these problems remain. In an attempt to resolve them, a comparison of hydrogen sulfide has been made with dimethyl sulfide (DMS) which is chemically similar, but less potent and can produce coma. To reduce the animals used, the cumulative method of Reed and Meunch (Am.J.Hyg. 27, 493,1938) was used to derive ED50 and LD50 values.

The intraperitoneal injection of DMS elicits a comatose state in Sprague-Dawley rats (200-300g body weight) that is dose dependent with an ED50 of 813 mg/kg. The period coma lasted less than 25 minutes and the animals appeared to be recovering fully. Animals that died so did several hours later of relatively abrupt respiratory failure. Using a 24-hour post-injection time mark, the LD50 for DMS is 537 mg/kg showing a decreased toxicity of greater than thirty fold as compared with hydrogen sulfide. It is noted that the LD50 is lower than the ED50 under these conditions.

Agents purported to have antidotal effects for sulfide poisoning, namely dithiothreitol (DTT) and sodium nitrite were studied for their ability to reverse coma and to prevent death. Sodium nitrite, 75 mg/kg administered at onset of coma decreased duration of coma, but DTT (50mg/kg) had no effect when administered at onset of coma or 20 minutes prior to DMS. Pretreatment with DTT caused a small reduction in lethality (LD50-661 mg/kg). Supported by EPA award CR 819550-01-GO.

In order to further characterize the neurotoxic effects of chronic Pb exposure, autoradiographic techniques were used to examine the effects of early Pb exposure on NMDA, PCP, and adenosine A1 receptors in neonatal rat brain. Rat pups nursed mothers exposed to 4% PbCO3 in their diet, or a Na2CO3 control diet from postnatal day 1 (P1) to P25. At P25, rats were sacrificed and the distributions of [3H]CGP 39653 binding to NMDA receptors, [3H]TCP to PCP receptors and [3H]CHA to adenosine A1 receptors in brain were assessed. Chronic Pb exposure was found to produce an increase (+20-30%) in the density of ligand binding to NMDA and PCP receptors in specific regions of the hippocampus. However, [3H]CHA binding to A1 receptors was found to be generally decreased (20-60%) throughout the neonatal rat brain. The present observations are consistent with recent behavioral and electrophysiological data indicating that Pb may directly alter the NMDA/PCP receptor complex in the rat forebrain and may also suggest a direct effect on inhibitory neurenomodulation via adenosine A1 receptors.

673.4 LEAD ELEVATES THE INTRACELLULAR CALCIUM ION CONCENTRATION OF NEURONS: A 19-NUCLEAR MAGNETIC RESONANCE STUDY OF NEURONS IN MICROCARTRIDGE CULTURE. P. A. X. Schanne, D. K. Barten* and J. A. Kessler, Depts. of Pediatrics, Pathology, Neuroradiology and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Exposure to lead (Pb) early in development leads to significant neurobehavioral deficits in humans and animals. The objective of this study was to determine if Pb on the intracellular calcium ion concentration [Ca2+]i of developing neurons. Neurons were isolated from the cortex and hippocampus of fetal rat brains on day 18 of gestation. Neurons were attached to Cytodex #1 microcarriers maintained in Eagle’s Minimal Essential Medium supplemented with 10% fetal calf serum at 37°C. [Ca2+]i was measured using a divalent cation indicator 1,2-bis(2-aminophenyl)-ethane-N,N,N’,N”-tetraacetic acid (SF-BAPTA) and the [Ca2+]i was measured using fluorescence. This method provides for the simultaneous identification and measurement of Ca2+ and a variety of heavy metals, including Pb2+. The untreated or baseline [Ca2+]i of neurons measured on days 4 to 6 in vitro ranged from 128 to 184 nM. Upon treatment with Pb2+ (5μM) the [Ca2+]i rose 50% to within 1 hr in cortical neurons and greater than 80% in hippocampal neurons. These data indicate that Pb2+ elevates [Ca2+]i, in developing neurons in culture. Given the importance of [Ca2+]i in the expression of neuronal structure and function we propose that these changes in [Ca2+]i may contribute to Pb-induced alterations in neural morphology, plasticity and responses to neurotransmitters.

673.5 SELECTIVE TOXICITY TO CENTRAL NORADRENERGIC NERVOUS SYSTEM IN POSTNATALLY LEAD EXPOSED RATS. S.G. Song, D.O. S. Seo, J.H. Chong, C.Y. Shin, M.U. Cho* and K.H. Ko. Department of Pharmacology, College of Pharmacy and Department of Chemistry, College of Natural Science, Seoul National University, Seoul 151, Korea.

Possibility whether postnatal lead ingestion can cause selective toxicity to central noradrenergic nervous system in rats was tested. Three groups of water rat: 1) Control, 2) Low dose and 3) High dose groups, were prepared. Right after parturition from dams rat pups received drinking water containing either 0% (control), 0.02% (low dose) or 0.2% (high dose) of lead acetate. At 2, 4, 6, and 8 weeks of age, dopamine β-hydroxylase (DBH) activity and Na-K ATPase activity were measured in 5 areas of rat brain; Telencephalon, Diencephalon, Limbriar, Pons/Medulla and Cerebellum. DBH activities were assayed by modified method of Coyle and Axelrod (1972) using S-adenosyl-[14C]methionine as substrate. DBH activity was determined as a criterion of lead poisoning to central noradrenergic nervous system and ATPase activity as a criterion of non-specific lead poisoning to any kinds of tissues. In lead exposed rats, DBH activities were higher but Na-K ATPase activities were lower than those observed in age-matched control animals. Selective toxicity of lead poisoning to central noradrenergic nervous system was evaluated by the changes of DBH activities without concomitant changes of ATPase activities. Brain areas where selective toxicity of lead seems to be induced were telencephalon and pons/medulla (2 weeks of age) and telencephalon, diencephalon and pons/medulla (4 weeks of age), midbrain and pons/medulla (6 weeks of age), cerebellum (8 weeks of age) in low dose group, and midbrain (6 weeks of age), cerebellum (8 weeks of age) in high dose group.


Astroglial cells accumulate Pb and are less sensitive to detrimental effects than neurons. A possible mechanism by which astrocytes adapt to Pb may be upregulating the expression of particular genes. We have previously demonstrated that exogenously added Pb resulted in enhanced synthesis of a 23kD acidic cytosolic protein by astrocytes. To begin to address the question of the mechanisms underlying the regulation of the 23kD protein by Pb, astrocytes were cultured in actioncinomycin D prior to the addition of the metal. Astrocytes, both pretreated or untreated, were exposed to 0, 5, and 50μM Pb acetate for 24 hours and pulse-labeled with [3H]-leucine. Proteins were separated by SDS-PAGE and subsequently analyzed by fluorography. Treatment with actioncinomycin D precluded synthesis of the 23kD protein. The differences in size and charge of the cytosolic proteins in Pb-exposed and untreated astrocytes were further characterized by two-dimensional electrophoresis. After a 24 hour treatment period with 0 or 50μM Pb acetate, cells were pulse-labeled and cytosolic proteins were analyzed by twodimensional electrophoresis and fluorography. The 23kD protein was resolved into 2 different charged species in Pb treated astrocytes. The untreated control revealed faint but definite spots corresponding to all but the most acidic of the 23kD proteins. Two spots corresponding to the 23kD proteins were notably more abundant in Pb-treated cells and an acidic trait of proteins ranging from 5.2-5.9pI was apparent in the same molecular weight region, thereby suggesting that the 23kD protein may accumulate and undergo post-translational modifications in response to Pb. These results suggest that Pb may affect both transcriptional and translational events in astrocytes.


A battery of in vitro assays was developed to detect the presence of neurotoxic metals, particularly Pb, in cultured astroglia. In order to produce such a battery, it was necessary to distinguish unique neurotoxic effects of Pb (a Pb signature) from the toxic effects of other compounds. The Pb signature we obtained was compared to the signature produced in astroglia by the epilepticogenic agent ibogaine (2). Cellular response to metal exposure were quantified by the use of vital fluorescent probes and interactive laser cytometry. We found distinct signatures for Pb and Fe. Whereas Pb stimulated an increase in intracellular glutathione content on day 6 of treatment, Fe had no effect. Furthermore, cell-cell communication via gap junctions, which was unaffected by Pb treatment, was increased in Fe-treated cells. In addition mitochondrial membrane potential was decreased in Pb treated cells but increased in Fe-treated astroglia. We are also comparing metal effects on Ca metabolism.

674.2 ONTOGENETIC ALTERATIONS OF ODC ACTIVITY IN DEVELOPING BRAIN REGIONS AFTER POSTNATAL EXPOSURE TO LEAD-ACETATE. N.H. Zawia* and G. J. Harry. DART/STB/NIEHS, P.O. Box 12233, RTP, NC 27709.

Onithiene decarboxylase (ODC), the rate limiting enzyme of the polyamine pathway, can serve as an important tool to examine the effects of toxicants on early developmental phases of the nervous system. ODC activity follows a distinct ontogenetic pattern in individual brain regions, peaking at periods of maximal cell proliferation. We have studied the effects of inorganic lead on characteristic developmental profiles of ODC activity in the cerebellum, neocortex and hippocampus. Two exposure models were used with Long Evans hooded rats: 1) lactational exposure via PND1 to PND20 (23 mg/kg daily dam dose); 2) daily dosing of pups by gavage (600 mg lead acetate/kg body weight) from PND2 to PND5. Animals were killed on PND 15, 20, and 20, and distinct brain regions were dissected, and then frozen at -70°C until assayed for ODC activity. Lactational exposure to Pb-acetate resulted in a dramatic (2 fold) stimulation of cerebellar ODC activity which was maintained up to PND 10 but dropped to control levels by PND 15. This induction of ODC activity was only observed in the cerebellum. In contrast to animals receiving low doses of Pb-acetate (lactational exposure), animals on direct Pb-acetate dosing exhibited an inhibition of ODC activity in all the brain regions examined. This loss of activity is suspected to be associated with high lead levels. These data suggest that low lead exposure can interfere with proliferative events in the postnatally developing cerebellum and suggest that lead may produce generalized perturbations in neuronal development.
These results suggest that immediate early genes may be involved in the granule cells of the facia dentata which are relatively immature at PND 4. However, it may be representative of a localization to the hippocampal gene response to TMT may be due to a differential regulation of these genes, a dramatic induction of c-fos mRNA in the hippocampus within half an hour. No changes in the expression of this gene were seen in the cerebellum following TMT-induced alterations, morphological, physiological, and biochemical, in the hippocampus, frontal cortex, and the cerebellum at 0.5, 1.5, and 5 hours following acute exposure to TMT (4 mg/kg, s.c.). In the neonatal (PND 4) rat hippocampus, the basal expression of c-fos, c-jun, and ODC was high and appeared to be unaltered at all time points examined following TMT injection. Two weeks after TMT treatment a marked loss of CA3 pyramidal neurons was seen in hippocampus, concomitant with a pronounced gliosis as visualized using BDNF protein IR and mRNA were abundantly expressed in pyramidal neurons as well as granule neurons of the dentate gyrus, as visualized using polyclonal antipeptide antibodies and oligonucleotide probes. Decreases in acetylcholinesterase IR was seen in the CA3 region. In addition, alterations in acetylcholinesterase (AChE) staining and choline acetyltransferase (ChAT) activity in the outer molecular layer (OML) of the dentate gyrus have been reported in TMT treated rats. Hydrgine (HYG) is used to attenuate some of the cognitive and behavioral deficits observed in conditions such as AD. HYG has been shown to affect the selective lesion of the hippocampus. The purpose of this study was to examine the effect of HYG on (1) TMT induced hyperactivity and (2) AChE staining in the hippocampus. Five adult male Long-Evans rats were assigned to 8 groups. Six groups were orally gavaged with 6mg/kg TMT chloride, three of these received one of 3 doses of HYG, 0.2, 1.2 or 3.0 mg/kg in an ethanol vehicle by gavage daily for 28 days. One was given vehicle, one handled and one TMT group untouched. The remaining rats served as controls with half being gavaged with vehicle. The animals were then observed in an open field for ten minutes a day for 3 days. Line crossings and rearings were noted. The rats were sacrificed and their brains sectioned at 20 μm for thionin or 40 μm for AChE stain. Statistical analysis of behavior and densitometry results indicate HYG reduced open field activity, and AChE stain density. These findings suggest that Hydrgine may be effective in treating damage by neurotoxins.

In the adult animal, the immediate early genes (IEG) such as c-fos, c-jun and other activity-dependent genes such as ornithine decarboxylase (ODC) are induced within minutes to perturbations to the cellular environment. We have examined the induction of these genes following acute exposure to a known neurotoxicant, trimethyltin (TMT). TMT-induced alterations, morphological, physiological, and biochemical, in selective neuronal populations in the hippocampus have previously been observed within 16-24 hours following an acute exposure. Using Northern blot analysis, we have examined the induction of these genes within the hippocampus, frontal cortex, and the cerebellum at 0.5, 1.5, and 5 hours following acute exposure to TMT (4 mg/kg, s.c.). In the neonatal (PND 4) rat hippocampus, the basal expression of c-fos, c-jun, and ODC was high and appeared to be unaltered at all time points examined following TMT exposure. However, in the adolescent (PND 35) rat, TMT exposure produced a dramatic induction of c-fos mRNA in the hippocampus within half an hour. No changes in the expression of this gene were seen in the cerebellum and the frontal cortex at either age. Expression of actin mRNA was not altered at TMT exposure at either age. The age-dependent immediate early gene response to TMT may be due to a differential regulation of these genes, however, it may be representative of a localization to the hippocampal granule cells of the facia dentata which are relatively immature at PND 4. These results suggest that immediate early genes may be involved in the response of the brain to TMT in an age-dependent and region-specific manner.
674.15

IN VITRO NEUROTOXICITY OF ORGANOTIN COMPOUNDS: T. A. Thompson, S. M. Togias, W. B. Severs* and M. L. Billingsley. Dept. of Pharmacology, Pennsylvania State University College of Medicine, HERSHEY, PA 17033

Organotin compounds induce neurotoxic changes: trimethyltin (TMT) causes selective patterns of neuronal destruction whereas triethyltin (TEL) causes degeneration of myelin. In order to characterize mechanisms of TMT and TET toxicity, human SMS-KCNR neuroblastoma, HTB-14 glioma and mouse 3T3 fibroblast cell lines were exposed to graded concentrations of TET (0-200 μM), TMT (0-200 μM) and SnCl2 for 48 hrs. Cell viability was determined using the fluorescent dyes calcine-AM and ethidium homodimer. TMT caused a dose-related loss of cell viability in SMS-KCNR cells (LD50= 25 μM); HTB-14 and 3T3 cells were resistant to TMT. In contrast, TET was toxic to SMS-KCNR (LD50= 7 μM) and HTB-14 cells (LD50= 10 μM). All cells tested were resistant to SnCl2.

Previous experiments coupling subtractive hybridization and molecular cloning demonstrate that a novel 88 residue protein, termed stannin, is expressed in TMT-sensitive cells. Immunoblot analysis indicated that SMS-KCNR cells, unlike hippocampus expressed stannin, while HTB-14 cells gave a background signal, suggesting that stannin expression correlates with TMT sensitivity. Transfection experiments, which cause stable heterologous expression of stannin in resistant cell lines are currently in progress.

674.16


Inorganic mercury (Hg^2+) is a recognized cytotoxican, however, the mechanism by which Hg^2+ entersthe cell has not been defined. Recently it has been reported that cations such as zinc, cadmium, and lithium are transported across cell membranes via the band 3 anion channel protein. Band 3 is a ubiquitously expressed protein that mediates the exchange of anions, maintains acid-base balance, and provides a cytosolic binding site for glycolytic enzymes. By using erythrocytes as a model, we have shown that DIDS, a specific band 3 inhibitor, blocks Hg^2+ uptake.

Erythrocytes were incubated at 37°C for 10 minutes in a solution containing 10 μM HEPES buffer, pH 7.4, 203Hg, increasing concentrations of HgCl2 (0.1 μM to 100 μM) and a range of inhibitor concentrations (10 μM to 200 μM). Hg^2+ uptake was inhibited in a dose-dependent manner with increasing concentrations of DIDS. At a concentration of 10 μM HgCl2 and 200 μM DIDS, the percentage of 203Hg associated with the cells was decreased by more than 50%. The results indicate that the band 3 protein is a transporter of Hg^2+, perhaps in the form of an anionic species such as HgCl^-.

The presence of band 3-like proteins in brain might represent the site through which essential and toxic elements enter cells in the central nervous system.

674.17


Using extracellular microelectrode recording techniques, population spikes (PS) and long-term potentiation (LTP) were recorded to examine the effects of methymercury (MeHg) on synaptic transmission in isolated hippocampal slices. PSs were induced in the CA1 region by stimulating (0.25 Hz) schaffer collaterals and LTP was induced by applying a brief high-frequency stimulation (HFS, 15 trains of 5 stimuli at 100 Hz in 100 ms intervals). MeHg was applied to slices acutely by perfusion with artificial cerebrospinal fluid (ACSF). At 20-500 μM, MeHg first increased PS amplitude by 20-50% and then decreased and blocked the PS completely. Time to increase and time to block PS were both concentration-dependent. In the absence of MeHg, application of HFS increased PS amplitude by 50-100%, an effect which lasted for at least 2 hours. With simultaneous application of 20-100 μM MeHg and HFS, the PS amplitude was increased further by 20-50% based on the already elevated PS amplitude by HFS. Subsequently, the PS amplitude was reduced and finally blocked in a similar way to that produced by MeHg on PSs recorded without HFS. If MeHg (10μg/mL) was applied for 20 min before HFS, PS amplitude was decreased below the control level. However, LTP was still induced by HFS, suggesting that MeHg may not affect induction of LTP even though it did suppress the maintenance of LTP.

Reversibility of effects of MeHg on PS and LTP were examined by washing slices with ACSF. At 20-500 μM MeHg, the PS amplitude decreased by 50% and increased further by 20-50% based on the already elevated PS amplitude by HFS. With increasing concentrations of DIDS (0-10 μM), the percentage of 203Hg associated with the cells was decreased by more than 50%. The results indicate that the band 3 protein is a transporter of Hg^2+, perhaps in the form of an anionic species such as HgCl^-.

The presence of band 3-like proteins in brain might represent the site through which essential and toxic elements enter cells in the central nervous system.

674.18

CUMmulative DOSE RESPONSE FUNCTION FOR LEAD ACETATE, TRIETHYLLICHLORIDE, AND METHYLMERCURY IN THE RAT HIPPOCAMPAL SLICE. S. B. Fountain* and J. D. Rowan. Dept. of Psychology, Kent State University, E. Lansing, KY 40386.

The present study assessed the effects of lead acetate (PbAc), triethyltin (TEL) chloride, and methylmercury (MeHg) chloride on rat hippocampal slice excitability using a cumulative dose response function (cDRF) procedure. Slices were prepared using standard techniques and maintained at the interface of a pool of artificial CSF and an O_2/CO_2 atmosphere. Stimulating and recording electrodes were positioned in the Schaffer collaterals and CA1 cell body layer, respectively. When a stable waveform was recorded, an input/output (I/O) profile was obtained as a baseline measure by administering a series of increasing stimulus intensities. Exposure was accomplished by switching from normal ACSF to agent-bearing medium reservoirs supplying the slice chamber. Tests of excitatory and inhibitory systems in area CA1 of the hippocampal slice were conducted using a paired-pulse technique (25 μsec interpulse delay) every 5 min over a period of 2 hr postexposure. During the monitoring period, the cDRF procedure monitored changes in excitatory and inhibitory systems beginning with a low dose of an agent, then successively higher doses were introduced every 30 min. Slices were exposed to 0.1, 1, 10, and 100 μM PbAc, TEL, or MeHg in successive 30-min periods. Slices were exposed to only one agent. Significant suppression of excitability was observed at the 10 μM dose of TEL and at the 100 μM dose of PbAc and MeHg. In addition, MeHg produced significantly greater suppression than PbAc at the 100 μM dose. The results favor the view that the hippocampal slice preparation may prove to be a valid model of neurotoxicity screening.

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NEUROTOXICITY: METALS—ALUMINUM

675.1 EFFECTS OF ALUMINUM ON CALCIUM REQUIREMENT MECHANISMS IN 7 DAY AND ADULT RAT BRAIN. W.R. Mundy1, P.R.S. Kodavanti2, V. Dolchin2 and H.A. Tilson. Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Aluminum (Al) is a neurotoxicant which acts on a number of targets in the central nervous system. Recent studies from our laboratory indicated that Al chloride interferes with Ca++-homeostasis in the cerebellum. We have further examined the effect of Al on cellular Ca++-homeostasis in the developing and adult rat brain. Ca+-uptake was examined in mitochondria (M) and microsomes (ER), and Ca++ ATPase activity examined in synaptosomes (SN) isolated from the frontal cortex, hippocampus, and cerebellum of 7 day (developing) and 5 month (adult) male, Long-Evans rats. Ca+-uptake was greater in the hippocampus and cerebellum of adult rats compared to developing rats, but was similar in the cortex. Al (50-800 μM) inhibited Ca+-uptake in M and ER at both ages. Developing rats were more sensitive to Al inhibition of Ca+-uptake (IC50 425-620 μM) compared to adult rats (IC50 600-1100 μM) in M. Al inhibition of Ca+-uptake was similar for both ages in ER (IC50 775-1500 μM). Ca++-ATPase activity was greater in SN from all three brain regions in adult compared to developing rats. However, Al-induced stimulation of Ca++-ATPase activity in the cerebellum was similar for both ages. These results suggest that some mechanisms of Ca++-homeostasis are not fully developed in the 7 day old rat, and that developing rats are differentially sensitive to the effects of Al compared to adults.

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